THE GERM-CELLS OF CICADA (TIBICEN) SEPTEMDECIM (HOMOPTERA).[1]

ELMER L. SHAFFER,

DEPARTMENT OF BIOLOGY, PRINCETON UNIVERSITY.

CONTENTS.

[1] A thesis presented to the Faculty of Princeton University, in candidacy for the degree of Doctor of Philosophy.

A. Introduction.

The study presented here was done at Princeton University during the last year. The material was collected during the appearance of the 17-year locust (*Cicada septemdecim*) in the vicinity of Princeton, N. J., during the spring of 1919 at the suggestion of Professor E. G. Conklin. With such an interesting life cycle as these insects have, it was thought that a cytological study of their germ cells might reveal some important facts in the history of the mitochondria and the chromosomes. Both of these are constant and important structures of all cells, and it is the opinion of the writer that no cytological study can be complete which neglects either one or the other. It is only by a correlated study of cytoplasmic and nuclear structures that we can ever hope to solve the many perplexing questions in cell economy. We use the almost mystic phrase: "interaction between nucleus and cytoplasm" in many cases to cover our ignorance concerning certain cell activities, but we are far from knowing any of the specific actions and reactions between the nucleus and cytoplasm. Within recent years the study of mitochondria of animal and plant cells has attracted many workers, with the result that many cytologists have come to regard these structures as of vital importance in cell activities. Attention, which for many years has been centered on the nuclear activities, has been drawn to a more intensive study of the cytoplasm and its structures. All these studies have emphasized the importance of such structures as mitochondria in relation to cell metabolism. Moreover, although the chromosome hypothesis of heredity seems to be firmly supported by a vast amount of evidence, yet one group of cytologists (Meves, Benda, Duesberg, etc.) maintain that the mitochondria also have a rôle as the bearers of hereditary characters. While the evidence which these workers have gathered is not of a convincing nature, nevertheless the facts are worthy of careful consideration. There are some reasons for believing that inheritance in some cases is through the cytoplasm, and we must not loose sight of the fact that "cytoplasm as well as nucleus is concerned in heredity and differentiation" (Conklin, '16). However, whether or not such cytoplasmic structures as

mitochondria constitute the idioplasm is an entirely different problem. Modern researches in genetics have shown that, despite mutations, hereditary characters are relatively stable and that the hereditary constitutions of organisms are definitely organized; hence the idioplasm which is causal in the development of the hereditary characters must similarly be stable and highly organized.

Keeping in mind the "cell as a whole," I have studied the chromosomes and mitochondria in the oögenesis and spermatogenesis of *Cicada*, and my observations give added evidence to the chromosomes as the idioplasmic substance, while there is no evidence from an unbiased standpoint that the mitochondria behave as idioplasmic substances. There is evidence, however, that the mitochondria are intimately concerned in cell metabolism.

Throughout this work I have had the constant advice and encouragement of Prof. E. G. Conklin, and it is with great pleasure that I here express my indebtedness.

B. Materials and Methods.

The youngest specimens of *Cicada* obtained were those of the second pupal stage about three weeks prior to their emergence from the ground and their final moult into the imago. These specimens were collected about the middle of April by digging under trees in the vicinity of Princeton. The pupæ were found lying about a foot from the surface of the ground and were most abundant several feet away from the tree trunks. In the testes of such pupæ, one finds most of the cells in the maturation stages besides an abundance of spermatids, spermatozoa, and a few spermatogonia. In the adult or imago, the testes are almost completely filled with sperm except for a small number of spermatogonia. After copulation, the testes are reduced to about one tenth their former size and contain only a small residue of spermatozoa, some degenerated cells and a few spermatogonia which also show signs of degeneration.

There are two testes, each consisting of a great many radiating ellipsoidal follicles which give the testes a berry-like appearance. In the female there are two typical ovaries, each consisting of a

great number of ovarian tubules containing a great many oöcytes. The oldest oöcyte of a second stage pupa is about one seventieth the linear size of the oldest oöcyte of the adult, which shows the tremendous growth that takes place in a few weeks. It has been estimated (Marlatt, 1898) that the female *Cicada* lays between 400 and 600 eggs.

The male and female gonads were dissected out in Ringer's solution and fixed in either Flemming's (strong), Bouin's, Benda's, or Regaud's fixing fluids. Mitochondria were well preserved by the Flemming, Benda and Regaud fluids, but were either partially or wholly destroyed in Bouin's fluid depending on the length of time the gonads remained in the fixing fluid. Material fixed in Flemming's fluid (10 to 12 hours) was usually the best for studying both chromosomes and mitochondria. Sections were cut 8 to 10 micra in thickness and the stains used were iron-hæmatoxylin (with and without counterstain), Benda's crystal-violet and alizarin, and Altmann's fuchsin-methylene green.

The developing eggs were also collected from time to time for a study of the chromosomes of the embryonic cells. These were fixed in either Bouin's or Carnoy's fluids and were imbedded by the celloidin-paraffine method.

C. Observations.

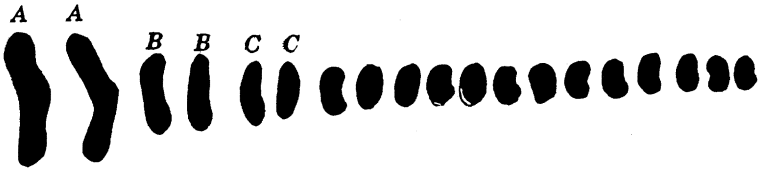
1. *Diploid Chromosome Groups.*

(*a*) *Spermatogonia.*—The spermatogonia are found in the proximal end of the ellipsoidal follicles of the testes. They form a cap of cells at this end of the follicle containing the primary and secondary spermatogonia. From the proximal end of the follicle there proceeds a short narrow filament which contains the spermatogonia of the multiplication stages. Mitotic figures are quite abundant among the spermatogonia of the multiplication stages, but the metaphase plates are usually so crowded that it is impossible to make accurate counts of the chromosomes. On the other hand, the primary and secondary spermatogonia rarely show cell divisions, but the metaphase plates when they appear are very clear.

The male chromosome number is 19, which indicates that there

is present an unpaired sex element (Figs. 1 and 2). One pair of the complex is strikingly larger than the rest, being in the form of somewhat curved rods (Text-fig. 1). This pair corresponds to the "macrochromosome" pair described by Kornhauser ('14) in *Enchenopa*.

Two other chromosome pairs (*BB*, *CC*, Text-fig. 1) can also be distinguished from the other chromosomes by their size, being approximately half the size of the macro-chromosomes (*AA*). The other 13 chromosomes show no size differences which would enable us to arrange them in pairs or distinguish



TEXT-FIG. 1. Spermatogonial chromosomes, showing the relative sizes of the chromosomes; the macrochromosome pair, *AA*, the *BB* and *CC* pairs, and 13 other chromosomes which show no size differences.

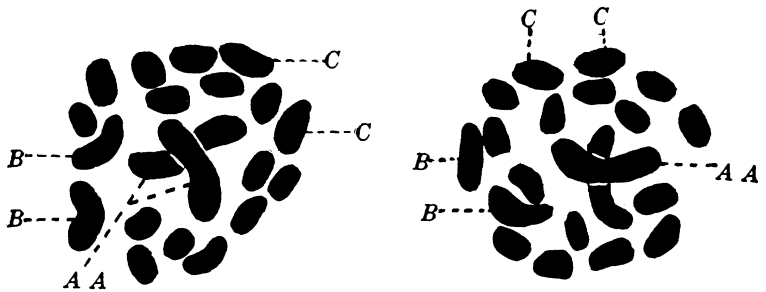
them from each other. However, as will be later shown, the *AA*, *BB*, and *CC* chromosome pairs are so characteristic in their form and size that they can be recognized in all the diploid groups. The size relations of the chromosomes of *Cicada* correspond to those described by Kornhauser ('14) in *Enchenopa*. Also in the *Cercopidæ* (Homoptera), Boring ('13) has described three pairs of chromosomes (*A*, *B*, *C*) which bear similar size relations to the three pairs here described in *Cicada*.

The sex-chromosome cannot be identified in the spermatogonial groups either by its size nor by any peculiarities in its staining reactions. In the resting spermatogonia there is, however, always present a single chromatin nucleolus (Fig. 13) which probably is the persisting sex chromosome.

(*b*) *Ovarian Follicle Cells.*—Among the follicle-cells of the ovary, mitotic figures are very abundant. I have found a great many mitoses not only among the follicle-cells surrounding the young growing oöcytes, but even among the follicle-cells surrounding the almost mature oöcytes. I have searched for evidences of amitotic division among these cells, which has often

been described, especially in the follicles surrounding mature oöcytes; but I have failed to find any strong evidence for the occurrence of this method of nuclear division. The only indication that amitotic division may take place is found in the follicles surrounding the old oöcytes. Here the cells are usually binucleate and appear to be the end stages of amitotic divisions. However, I have found many karyokinetic figures among such cells and it seems reasonable to suppose that the binucleate follicle-cells arise through the failure of the division of the cell body following mitotic division of the nucleus.

All metaphase plates of dividing follicle-cells show 20 chromosomes (Figs. 3, 4, 5, 6, Text-fig. 2), and it is possible in all of these to recognize the chromosome pairs *AA*, *BB*, and *CC* as in the spermatogonial plates, by their size relations. Figure 12 is that of the late telophase of a dividing follicle cell in which



TEXT-FIG. 2. Two metaphase plates from ovarian follicle-cells showing the *AA*, *BB*, *CC* pairs of chromosomes; note that the *AA* pair lies in the center of the group.

one of the macrochromosomes (*A*) can be recognized in the daughter cells. It will also be seen that there is present a precocious longitudinal split of the chromosomes in preparation for the next cell-division.

In the follicle-cells surrounding the older oöcytes, the chromosomes do not have the thick compact appearance found in the younger follicle-cells. The chromosomes are usually thinner, poor in chromatin content, and the respective chromosomes appear somewhat longer, often showing an equational split (Figs. 10, 11). I am unable to account for this difference in appearance of the chromosomes (compare Figs. 7, 10, 11). In

the resting nuclei of the old follicle-cells, it is very noticeable that they are very poor in chromatin content, all the basichromatin being accumulated in one or two small masses (nucleoli). When such cells prepare for mitosis, the chromosomes which are reconstituted are correspondingly poor in chromatin. However, the linin basis of the chromosomes is still present and consequently the number and size relations of the chromosomes is maintained. This, I believe, gives added evidence to the view previously expressed (Shaffer, '20) that the linin is the morphologically stable substance which maintains the chromosomal organization and structure.

(*c*) *Embryonic Cells.*—Although a number of the developing eggs of *Cicada* were collected, I was unable to obtain good material for a study of the chromosomes due to the difficulties in sectioning. The eggs were so full of yolk that it was possible to cut them only by imbedding by the celloidin-paraffine method.

Figure 9 is that of a metaphase plate of a cell from the blastoderm showing 20 chromosomes, and hence of the female type. The chromosome pairs *AA*, *BB*, and *CC* can be distinguished as in the other diploid nuclei. While I have been unable to make an exhaustive study of the chromosomes of the embryonic cells, yet I have found no variations in the chromosome numbers or in their size relations.

(*d*) *Somatic Cells.*—On dissecting the female locusts to remove the ovaries, a number of round, brown-pigmented bodies resembling eggs were found in the abdomen. On sectioning these it was found that they were of a glandular nature and are perhaps concerned in the secretion of adhesive materials for the eggs. In a small cap of cells which lies at one end of these glandular bodies, mitotic figures were found in abundance. There are always 20 chromosomes which show similar size relations to the diploid chromosome groups previously described. After mitotic division of the nucleus the cell-body fails to divide, resulting in the formation of binucleate and multinucleate cells. At the time of division of such multinucleate cells, typical triasters are formed (Fig. 52). In the telophase of such a division, the tripartite daughter nuclei reconstruct to form six separate nuclei, the cell-body again failing to divide. At this time the

cytoplasm becomes active in the secretion of large globules and although the nuclei increase in number, I have been unable to find mitotic figures and it seems possible that the increase in their numbers is brought about by amitosis.

2. *Spermatocytes.*

(*a*) *Growth Stages.*—Unfortunately the material which was collected showed very few of the early growth stages of the spermatocytes and consequently I was unable to make a detailed study of the process of synapsis. Apparently the early growth stages must take place some time before the month of April. In the youngest pupæ which I have collected, the only growth stages of the spermatocytes which I have been able to find are those of the pachytene-bouquet stage (Fig. 17) in which the thick synaptic threads are polarized at one side of the nucleus. Sections across the bouquet usually show 18 chromatic blocks representing the end view of the threads. Since each loop has been cut twice, this would indicate that there are 9 pachytene loops. At the base of the polarized bouquet is usually found the compact, deeply-staining nucleolus (Fig. 17, *X*) which is the persisting sex-chromosome. This is undoubtedly the same compact chromatic nucleolus found in the resting stages of the spermatogonia (Fig. 13). As is usual, the bouquet is polarized toward the pole of the cell containing the idiozome (Fig. 17, id.). It has often been stated that the idiozome exerts some attraction on the synaptic threads influencing their polarization. There is no evidence to support this view and I am inclined to believe that the same factors which determine at which point in the cell the idiozome should lie also determines the polarization of the synaptic threads.

Occasionally the pacyhtene threads show a longitudinal split, and in such cases it is noticed that the chromatic granules (chromomeres) of the two halves of the thread do not correspond either in size or location. Consequently the longitudinal split cannot be interpreted as an equational split, but is rather the primary split or point of synaptic union. Usually there is present a single loop of the bouquet which is much larger than the other loops (Fig. 17, *AA*) and this undoubtedly represents

the synaptic condition of the macrochromosome pair of the spermatogonial chromosomes.

(*b*) *Tetrads and Maturation Divisions.*—Stages of the early prophases of the spermatocytes were quite abundant and it was possible to follow the formation of the first maturation tetrads. In the early prophases, the homologous threads show a great variety of twisting about each other (Fig. 20). One pair (*AA*, Fig. 20) is easily distinguishable from the others by its large size and is derived from the spermatogonial macrochromosomes. Figure 18 represents the stages in the formation of the definitive tetrad from this pair (Figs. 61, 62, 63, 64). In the early prophases the homologous threads of the *AA* tetrad are very long and twisted about each other. However, they retain their connections at the ends, thus making the tetrad a large ring if its twists were straightened out. The space enclosed by this ring is the interchromosomal space which marked the point of synaptic union of the threads. In the later prophase stages, the large ring condenses, the threads become thicker retaining their point of union at the ends and the interchromosomal space becomes reduced in size until in the definitive maturation tetrad it is reduced to a small oval slit between the two halves of the ring (Figs. 18, 21, 62). In the first maturation metaphase, the macrochromosome tetrad no longer appears in the form of a ring, but rather in the form of a ring flattened at the poles, or as two slightly bent rods whose concavities oppose each other. In a similar way, the tetrad derived from the *BB* pair of the spermatogonia goes through the formation of a ring tetrad (Fig. 19), resulting in a tetrad similar to the macrochromosome tetrad (*AA*), but approximately half its size. The other tetrads show no ring formation; usually the homologous threads become free at one of the synaptic ends, retaining their connection at the other end, thus giving the appearance of two chromosomes joined end-to-end (Fig. 20). The condensation of such tetrads produces the typical dumb-bell form tetrad, with the narrow portion of the dumb-bell marking the retained point of synaptic union. Thus, if the point of synaptic union is retained at both ends, rings like the *AA* and *BB* tetrads are produced; if the synaptic union is retained at one end only, the dumb-bell type

tetrad is produced. Payne ('14) has described a different method of ring-formation in *Forficula*. The two univalent chromosomes are first joined end-to-end; while retaining this point of union, the free ends come together by a bending process with the resulting formation of a ring each half of which represents a univalent chromosome. A similar method of ring-formation has been described by Sutton ('02) in *Brachystola* and by Davis ('08) in several Orthopterans. In *Forficula* besides the "bending process" of ring-formation, Payne describes ring-formation of the type here described in *Cicada*.

(*c*) *Maturation Divisions.*—In the metaphase plates of the first maturation division, the chromosomes are always grouped in a characteristic manner (Figs. 23, 24, 53 to 57). There are 10 chromosomes in the metaphase plate, 8 of which are arranged in a circle surrounding the macrochromosome tetrad (*AA*, Fig. 23) which always lies in the center of the group. The sex chromosome (Fig. 23, *X*) always lies outside this circle of chromosomes and often does not lie in the same plane. Boring ('07) has figured the chromosomes of a number of species of Homopterans in which the sex-chromosome lies outside the group of autosome tetrads. The constant position of the *AA* tetrad in the center of the spermatocyte complex can be traced back to the diploid chromosome groups in which the macrochromosome pair shows a marked tendency to lie in the middle of the metaphase plate with the other chromosomes grouped about them (text-fig. 2). In a previous paper (Shaffer, '20) it was pointed out that the characteristic grouping of the chromosomes in the metaphase had its explanation in the persistence of the interchromosomal linin fibres (Fig. 24) which undoubtedly persist as a part of the chromosomal architecture and maintain definite spatial relations between the chromosomes. The evidence in *Cicada* seems to support this view.

In the side-view of the metaphase, the *AA* and *BB* tetrads are arranged on the spindle in the direction of the spindle axis. Hence in polar views only a half of each tetrad can be seen (Fig. 23). In both the *AA* and *BB* tetrads the spindle fiber attachments are median (Figs. 18, 19) or atelomitic (Carothers, '14), and since the tetrads lie in the direction of the spindle axis, they

will separate in the anaphase at the point of synaptic union and the division is reductional. The other tetrads have terminal spindle fiber attachments, but I am unable to say whether they divide reductionally or equationally. In the case of the *CC* tetrad, there is evidence that the narrow portion of the dumb-bell actually marks the point of synaptic union, and since separation of the dyads in the anaphase occur at this point, the division is also reductional.

The sex-chromosome (Fig. 25, *X*) usually lies on the outer surface of the spindle. In the anaphase it usually lags behind the other dividing chromosomes, sometimes appearing bipartite, and passes undivided to one of the daughter cells (Figs. 27, 70). As the dyads come into the late anaphase of the division, a secondary (equational) split can often be seen (Fig. 27, *AA*).

Following the first maturation division, there is no interkinesis or construction of a telophase nucleus. The dyads again become arranged in the metaphase, each showing the secondary split. Figure 28 (also Fig. 58) is that of second spermatocytes (daughter plates), one having 9 dyads the other having 9 dayds plus the X-chromosome. It will be noted that the grouping of the dyads is exactly similar to the grouping of the tetrads in the first maturation division metaphase, namely 8 dyads arranged in a circle around the macrochromosome dyad. In *Notonecta*, Browne ('16) found that the chromosomes always assumed a definite grouping in the metaphase, but the grouping was different in the two maturation divisions. In the anaphase of the second maturation division all the dyads divide and there are no lagging chromosomes.

(*d*) *Giant Spermatocytes.*—It is quite common to find spermatocytes with double or more the number of chromosomes. Figure 59 is a photograph of such a spermatocyte in the metaphase which has over twice the normal number of tetrads. These giant spermatocytes develop normally and give rise to giant spermatids and spermatozoa (Figs. 31, 33*c*). The origin of these giant cells may be traced back to the spermatogonia in which there has been a failure of division of the cell-body resulting in cells with double the diploid number of chromosomes. Such cells are quite common among the spermatogonia of the multi-

plication stages. Wilcox ('95) has described giant spermatids and spermatozoa in *Cicada tibicen;* he also finds that they are derived from abnormal spermatogonia in which there has been a failure of the division of the cell-body, and that their development insofar as the spermatozoan is concerned follows a normal course with the production of typical spermatozoa which merely differ from the normal ones by their large size. It seems difficult to ascertain the significance of these giant cells or whether there is any possibility that they play a part in the fertilization of the egg.

3. *Growth Stages and Synapsis in Oöcytes.*

While it has not been possible to study the process of synapsis in the spermatocytes due to the absence of the proper stages in my material, in the oöcytes I have been able to follow the various growth stages in some detail.

The young oöcytes in varying stages of growth are found at the base of the nurse chamber or "Keimlager" (Fig. 34). They are always distinguishable from the nurse-cells by their definite cell outline, by the thread-like appearance of the chromatin as compared to the granular nuclei of the nurse cells, and by the presence of a definite mitochondrial zone. Only occasionally could oögonial divisions be found in the ovaries of the youngest pupæ, and hence I have been unable to trace the chromosomes from the last oögonial division into the early growth stages. The very early growth stages (leptotene, etc.) are not found in the material collected after the latter part of April but in the material collected earlier, there is an abundance of oöcytes in the pre-synaptic stages.

Figure 35 is that of the nucleus of a young oöcyte corresponding to von Winiwarter's "protobroque" nucleus. The chromatin is in the form of a network with the nodal points of the net staining somewhat more deeply than the rest of the reticulum. On closer analysis of this nucleus it is seen that the net-like appearance of the chromatin is due simply to the optical effect of numerous delicate chromatic threads crossing each other in all directions. In the later stages the individual threads become more evident and this stage (Figs. 36, 74) no doubt corresponds to von Winiwarter's "deutobroque" nucleus. Gradually these threads as-

sume more and more a definite individuality, and the nucleus then becomes filled with a number of very delicate leptotene threads (Figs. 37, 75). These often show a distinct polarization, usually being attached at one side of the nuclear membrane with the free ends suspended in the nuclear sap. During this stage there is also a marked tendency for the leptotene threads to become associated in pairs and this pairing becomes more marked in the later stages (Fig. 38). The threads are now markedly polarized and it becomes quite clear that they are actually pairing side-to-side (zygotene stage). This paired appearance of the threads is not an accidental one, for I have observed it in a great many cells with great clearness. That it is also not due to a longitudinal split of a single thread is evidenced by the fact that the chromatin granules in homologous threads do not lie at the same level. After this pairing has taken place and the threads have become well polarized, they gradually become shorter and thicker (Fig. 39), forming the typical pachytene stage, the threads sometimes showing the primary split or point of synaptic union. A typical pachytene bouquet stage follows, in which the loops have both their free ends attached at one pole of the spindle (Figs. 40, 76). Usually there is one especially large loop (*AA*, Fig. 40) similar to the large loop found in the bouquet stage in the spermatocytes, which undoubtedly represents the macrochromosome pair. Cross-sections of cells in the bouquet stage show the threads on end view (Figs. 41, 77), and it is possible to count these. Usually there are 20 such cut ends of the pachytene threads and since each thread has been cut twice, we can deduce that there are ten pachytene loops, and it is at once seen that this number corresponds to the reduced number of chromosomes.

The release from the bouquet stage sets free the thick woolly-looking pachytene threads (Fig. 42) and the primary split comes clearly into evidence, and often the homologous elements become separated along the synaptic line. As this separation continues, the homologous threads become twisted about each other assuming the typical strepsistene condition (Fig. 43). The separation may begin at either or both ends of the threads or they may retain their union at the ends and separate along their

middle points. As the strepsistene stage advances, the threads lengthen considerably, become more lightly staining, and as they separate more widely their individuality becomes less and less distinct. In the older oöcytes, the chromatin of the germinal vesicles appears more or less granular, much of it still retaining its affinity for the basic stains. On close analysis of such nuclei (Figs. 46, 47) much of the chromatin shows evidences of still retaining, in part, the arrangement in threads, and I interpret these as being the original conjugating threads of the early growth stages which have been greatly expanded.

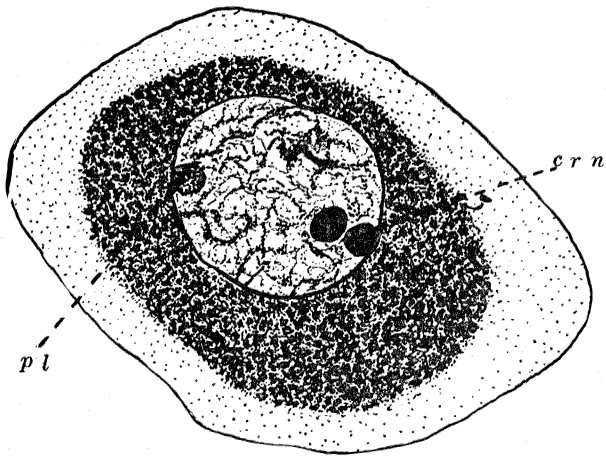
4. *Chromatin Nucleoli.*

I shall not attempt to review the literature concerning nucleoli since there is considerable confusion of interpretation in this field. It is evident that many of the different opinions expressed in the literature have arisen from the fact that these structures are not always homologous. Recently Nakahara ('18) has advanced the view that in the oöcytes of *Perla*, the nucleoli are derived from the yolk-nucleus which consists of a dense granular mass in the cytoplasm closely applied to the nucleus. As will be shown later, Nakahara's "yolk-nucleus" is really an accumulation of mitochondria, and it is difficult to see how these could give rise to nucleoli.

In the oöcytes of *Cicada* true chromatin nucleoli are found, and whenever they are present take the basic stains. The interest connected with chromatin nucleoli in the oöcyte is bound up with the possible homology of such structures with the chromosome nucleoli or persisting sex-chromosomes found in the growth stages of the spermatocyte. Since one sex-chromosome of the female is derived from the sperm, there is no à priori reason why we should not expect to find such a body persisting in the stages in the oöcyte homologous to those stages in the spermatocyte where it is found to persist as a nucleolus.

In the very young oöcyte of *Cicada* (Figs. 35, 36, 73, 74), there are usually present two chromatin nucleoli. In the leptotene nuclei these are no longer found, nor can they be found in the succeeding pachytene stages. However, in the strepsistene nuclei, two deeply staining bodies again appear (Figs. 43, 78)

which are similar to those found in the protobroque and deutobroque (preleptotene) nuclei. In the later stages these bodies become closely associated (Text-fig. 3, Figs. 81, 84) and persist as definite chromatin nucleoli. A true plasmosome is also present in these stages which becomes vacuolated and grows in size with the age of the oöcyte (pl., Figs. 44, 46). In the older germinal



TEXT-FIG. 3. Oöcyte of post-synaptic period, showing mitochondria arranged in perinuclear zone, and two chromatin nucleoli (*cr.n.*) and a plasmosome (*pl.*).

vesicles, the two chromatin nucleoli may persist (Fig. 87), but more commonly a single large chromatin nucleolus is found together with six to eight smaller nucleoli (Fig. 46).

It is difficult to say whether the two chromatin nucleoli really represent persisting sex-chromosomes and are therefore homologous to the chromosome nucleoli of the spermatocytes. There is some evidence that this is the case. The disappearance of the definite nucleoli during the synaptic stages (leptotene and pachytene) leads me to believe that they are resolved into chromatic threads which go through a synaptic process similar to the autosome threads. The fact that there are ten pachytene threads in the bouquet stage indicates that all the chromosomes are in synapsis. The reappearance of the two chromatin nucleoli in the strepsistene stages are perhaps brought about by a re-condensation of the synaptic threads representing the sex pair resulting in the formation of the two compact chromatin nucleoli.

This view is supported by the fact that in those stages where the nucleoli re-appear, they usually lie close to each other (Figs. 43, 78), and in the strepsistene stages they are more loosely granular in appearance than in the later stages. The later increase in number of the nucleoli in the germinal vesicle stages may be an expression of the increased metabolic activity of the nucleus during this period.

Wilson ('06) was unable to find chromosome-nucleoli in the oöcytes of *Anas*, *Euschistus* and other forms during the "contraction figure of the synaptic period" and he is inclined to doubt the persistence of nucleoli in the oöcytes homologous to those found in the spermatocytes. As has been shown in *Cicada*, it is quite probable that the sex-chromosomes go through synaptic stages just like the autosomes and hence do not persist as compact bodies in these stages as does the odd chromosome of the spermatocytes. However, they may be present in the form of nucleoli before and after the synaptic period. Foot and Strobell ('11) have described a chromatin nucleolus in the oöcytes of *Protenor* which gives rise to two large idiochromosomes at the time of cell-division. In *Gelastocoris*, Payne ('12) has described a chromatin nucleolus which appears after the last oögonial division and persists until shortly after synapsis. It later becomes reduced in size and disappears, and Payne interprets it as having been derived from the sex-chromosomes. The reason for the persistence of the sex-chromosome in the spermatocyte and its non-persistence in the oöcytes during synapsis may lie in the fact that in the former the odd sex element has no homologue with which to pair, while in the latter the two sex-chromosomes are homologous and synapsis becomes possible. However, with the wide variations which these nucleolar structures exhibit in the oöcytes, it is not possible to make any generalization as to their homology with the nucleoli of the spermatocytes.

5. *Discussion of the Chromosomes in Homopterans.*

(*a*) *Review.*—The earliest work on the chromosomes of the Hemiptera homoptera is that of Wilcox ('95) on *Cicada tibicen* in which he states that there are 12 chromosomes in the spermatogonia and 24 "elements" in the spermatocytes. The work of

Stevens ('05*a*, '05*b*) and of Morgan ('08, '09) on the *Aphids* and *Phylloxerans* has dealt mainly with the problem of sex determination. It was found that only spermatozoa bearing the sex-chromosome are functional and consequently only females are produced from fertilized eggs. Males are produced parthenogenetically from eggs which during the maturation processes reduce the chromosome number to one half the somatic number; hence there is only one sex-chromosome remaining in the male. Stevens ('06) and Boring ('07, '13) have studied the chromosomes of over 20 species of Homopterans mainly in relation to sex determination. Kornhauser ('14) has made a comparative study of the chromosomes of two species of *Enchenopa* in which he describes parasynapsis and pre-reduction.

In all the Homoptera studies thus far with one exception, the sex-chromosomes consist of but a single (X) element which persists as a chromosome nucleolus through the growth stages of the spermatocyte and passes undivided to one pole of the first maturation spindle. The only exception to this is that described by Kornhauser ('14) in *Enchenopa binotata* in which there are two sex-chromosomes (XY). These behave very much like the autosomes in the growth stages of the spermatocyte, becoming resolved into synaptic threads and pairing in synapsis much as the autosomes do. In the preleptotene stages the sex pair is found persisting as nucleoli. In the strepsistene stages, when the chromosomes become more diffuse and lightly staining, the chromatic nucleoli again appear by a condensation of the threads representing the sex pair. It will at once be noted that this behavior of the sex pair in *Enchenopa* resembles the behavior of the two chromatic nucleoli which I have described in the oöcytes of *Cicada* and I am, therefore, led to believe that the chromatic nucleoli of the oöcytes are homologous to the chromosome-nucleolus of the spermatocytes.

A common characteristic of the diploid chromosome complexes of many Homopterans is the usual presence of a pair of large rod-shaped chromosomes (*AA* pair of *Cicada*). Stevens ('06) has shown these in *Aphrophora*, and Boring ('07) has figured these in many of the Homoptera that she has studied. Kornhauser ('14) had described them in two species of *Enchenopa* that he has

studied and applies the name "macro-chromosome" to them. Besides the macrochromosome pair, there are usually present one or two chromosome pairs (the *BB* and *CC* pairs of *Cicada*) which are somewhat smaller than the macrochromosome pair, but which can easily be distinguished by their size from the other chromosomes. The presence of a pair of macrochromosomes and one or two pairs of somewhat smaller chromosomes therefore seems to be characteristic of the diploid chromosome groups of the Homoptera.

(*b*) *Ring Tetrads.*—McClung ('14) has described two types of ring tetrads in the Orthoptera which he terms (1) the *Hippiscus* type, and (2) the *Stenobothrus* type. Both types of ring tetrads are essentially the same as far as the formation and relation of their elements (chromatids) are concerned. Half of each ring represents a whole chromosome and each univalent chromosome is secondarily split, so that each ring tetrad really consists of two rings superimposed one upon the other. The difference between the two types lies in their relation to the spindle-fiber attachments and their position in the metaphase plate. In the *Hippiscus* ring type, the spindle-fiber attachments are at the synaptic ends (terminal) and the rings lie flat in the metaphase plate. Consequently polar views of the metaphase plate show the entire ring, while side views show only half of the ring. In the anaphase the two superimposed rings separate and consequently the division is post-reductional. On the other hand, the rings of the *Stenobothrus* type usually have median spindle-fiber attachments and the ring lies in the metaphase in the direction of the spindle axis. Only lateral views of the spindle would therefore show the complete ring, while polar views show only half of the ring. In the anaphase the two halves of the ring (which are univalent chromosomes) separate as simple V's and the first maturation division is pre-reductional.

In *Cicada*, the large ring of the early prophases is derived from the macrochromosome pair, *AA*, of the spermatogonia. In the spermatogonial divisions this pair has median spindle-fibre attachments and the spindle-fiber attachments of the ring tetrad derived from this pair are also median (Fig. 18). In the metaphase the ring is arranged on the spindle in the direction of the

spindle axis with the sister chromatids directed toward the same pole. Polar views of the metaphase (Fig. 55) show only half the tetrad, but oblique views show the relation clearly (Fig. 57). Thus the rings of *Cicada* are of the Stenobothrus type and they divide reductionally in the first maturation division.

(*c*) *Synapsis.*—Although I have been unable to study the process of synapsis in the male germ cells, my observations on the oöcytes indicates that the chromosomes conjugate parasynaptically, and there is no reason for supposing that it might be different in the spermatocytes. From Fig. 38 it is quite evident that the leptotene threads pair side-to-side, but the essential point is whether each leptotene thread actually represents a single univalent oögonial chromosome. Wenrich ('16) has shown in *Phrynotettix* that the leptotene threads are derived from the telophase chromosomes of the last spermatogonial division by a process of unravelling of the chromatic blocks contained within separate chromosomal vesicles. In *Cicada* I have been unable to trace the oögonial telophase chromosomes into the early growth stages of the oöcyte, but there is some indirect evidence that each leptotene thread is derived from a single oögonial chromosome. In the late leptotene stages, the threads are well polarized and all extend in one direction through the nucleus. I have studied a great many such nuclei in cross-section and it is possible to count the number of leptotene threads cut on end view. Such counts usually approximate the diploid chromosome number (20) and points to the fact that each leptotene thread is derived from a single chromosome.

As has been noted before (page 417), a casual study of the germinal vesicles of the oöcytes does not indicate any persisting individuality of the chromosomes; however, careful and minute analysis of such nuclei reveals the fact that the synaptic threads are still present but in a much-expanded and diffuse condition. Similarly in the male germ cells following the strepsistene stages, the synaptic threads appear to loose their individuality, becoming diffuse and widely expanded ("confused stage" of Wilson). This diffuse stage in the spermatocytes, although of relatively short duration, is no doubt homologous to the "germinal vesicle" stage of the oöcyte, since both occur at homologous periods in

the male and female germ-cells, namely, after synapsis and before the appearance of the definitive maturation tetrads.

D. Observations on Mitochondria.[1]

1. *Mitochondria in Spermatogenesis.*

(*a*) *Spermatogonia.*—Mitochondria are present in the spermatogonia of all stages in the form of granules usually localized at one end of the cell, usually the end directed toward the cyst cavity (Fig. 13). This is quite the usual location of mitochondria in spermatogonia, but as I have shown in *Passalus* (Shaffer, '17), the mitochondria may be diffusely spread in the cytoplasm. There is no indication that the mitochondria of the spermatogonia ever assume the form of filaments. During mitosis the mitochondria are spread along the outer spindle-fibers and by the time of the telophase and the cell-constriction, the mitochondria become grouped as granular masses lying above the daughter nuclei.

The amount of cell-degeneration taking place in the testes of *Cicada* was so striking as to call for more than mere casual observation. Sometimes an entire half of a follicle would be filled with cells in various stages of degeneration. This degeneration is usually found only among the spermatogonia prior to the commencement of the growth stages of the spermatocyte, which seems to be a critical time for the cells. Once they begin the synaptic processes, they apparently go through to maturation normally. It is quite noticeable that cell-degeneration is more abundant in the adult testes than in the testes of pupæ.

[1] I shall continue to use the term "mitochondria" to denote those cytoplasmic structures, regardless of their form, which are preserved by special reagents (destroyed by acetic acid, etc.) and which take specific stains. I see no advantage in the employment of the term "chondriosome" as recently urged by Duesberg ('19). The term "mitochondria" is not only more commonly used in this country, but also conveys the idea of their nature quite as well as "chondriosome." It can be equally well applied to those structures when filar in form as when granular and it seems to be etymologically as proper as the term "chondriosome." The various names which have been proposed to designate these structures (chondrioconts, plastosomes, plastidules, karyochondria, etc.) are based either upon their supposed origin or else upon their morphology. Since there is still much confusion as to their origin and since their morphology may vary at different times in the cell-cycle, it seems best not to commit ourselves to the use of new terms but to employ the original name, "mitochondria," of Benda.

In studying the process of degeneration it is at once evident that the mitochondria are concerned in the process. The first noticeable change in the degenerating cells is that the mitochondria become larger and more deeply staining and the nucleus often becomes polymorphic (Fig. 14). While the mitochondria have increased in size, they have decreased in numbers and it is quite evident that the large mitochondria have grown in size by an agglutination of the smaller normal ones. I have described a similar process in the degenerating spermatogonia of *Passalus* (Shaffer, '17), but was unable to follow in detail the succeeding stages of degeneration. In *Cicada*, the further processes in the degeneration involve a continued agglutination or coalescence of the mitochondria which results in the formation of large bodies of globular form staining intensely (Fig. 15). This process is continued (Fig. 16) until finally the degenerated cell becomes a deeply staining mass with the nucleus barely visible. Often the large globules show vacuoles which I interpret as being due to a partial dissolution of their substance by the reagents used.

Scott ('16) found that in experimental phosphorus poisoning of white mice the mitochondria are the first elements of the acinus cells of the pancreas to show any pathological changes. They begin to loose their characteristic filamentous form and finally agglutinate in large compact masses in the cell. "The mitochondria in these agglutinated masses fuse to form droplets possessing the characteristic properties of lipoids" (Scott,'16, p. 251), hence developing a fatty degeneration of the pancreas. The significance of these observations of Scott is at once apparent in connection with the degeneration of cells above described in *Cicada*. The behavior of the mitochondria in the degenerating spermatogonia of *Cicada* is essentially similar to their behavior in the fatty degeneration of the pancreatic cells and I am consequently of the opinion that the degeneration of the spermatogonia is a fatty degeneration.

Recently, Athias ('19) has studied the mitochondria in the interstitial cells of the ovary of the bat, *Vespertilion idæ*. The mitochondria are present here in the form of granules and filaments, which gradually become transformed into fat globules.

These represent "sans doute le produit de sécrétion des cellules interstitielles." Athias is of the opinion that the mitochondria are transformed into lipoid globules by a change in their chemical constitution. "On observe tres souvent des images qui montrent que le produit graisseux doit resulter d'un changement chimique de la substance mitochondriale; on peut suivre, en effet, les differentes phases de la transformation des plastosomes en gouttelettes de graisse" (page 195).

What the significance of such cellular degeneration, as found in the testes of *Cicada*, can be is difficult to say, since it occurrs regularly and is moreover found in almost all insect testes. It may possibly be that these degenerated cells in some way supply nutriment to the spermatozoa, as suggested by Wieman ('10) in the case of degenerated cells in the testes of *Leptinotarsa*, and are hence homologous to the nurse cells of the ovaries. I have often noticed deeply staining bodies, somewhat resembling the degenerated cells, lying among the spermatozoa; but other than this there is no evidence that the degenerated cells are a source of nutriment for the spermatozoa.

(*b*) *Spermatocytes.*—Since the spermatocytes of the early growth period are not present in my material, it has been impossible to follow the mitochondria from the last spermatogonial division into the spermatocytes. However, in the pachytene stages (Fig. 17) mitochondria are present in the form of filaments which sometimes appear granular. As will be noticed they are most abundant at the pole of the cell where the idiozome is located, which is the usual localization of the mitochondria in the spermatocytes, but which is by no means general. In *Passalus* (Shaffer, '17) I have found that the mitochondria of the early spermatocytes are in the form of diffusely spread granules and are often most abundant in a zone immediately surrounding the nucleus.[1] Undoubtedly, the filar mitochondria

[1] Duesberg ('18) has questioned these observations stating (foot-note, p. 138): "it is characteristic, even if not quite general, that the male auxocytes have their chondriosomes accumulated at one pole of the nucleus around the idiozome." Duesberg's ('10) own figures (Figs. 51, 52, 53) of the spermatocytes of the guinea-pig seem to contradict this, for he describes the mitochondria as being spread diffusely in the cytoplasm. "Ils (mitochondria) cessent d'etre groupes exclusivement autour de l'idiozome, pour se repandre dans toute le cytoplasme" (p. 65).

of the spermatocytes are genetically related to the granular ones of the spermatogonia. There is no evidence that the mitochondria go through a process of pairing during the growth stages; on the contrary they increase in numbers. This increase in the numbers of the mitochondria is a most important point in an understanding of their nature and significance. Such an increase in numbers of mitochondria during the growth period has been explained by some workers (Goldschmidt, Buchner, etc.) as being due to an elimination of material from the nucleus into the cytoplasm (chromidial theory). On the other hand, another group of workers insist that mitochondria are always derived from pre-existing mitochondria by a process of autonomous division. Wilke ('13) has described such a process in *Hydrometra*. As will be shown later, it is quite possible that the mitochondria may arise in another way.

As the first maturation spindle is forming (Fig. 21), the mitocondria begin to migrate from one pole of the spindle towards the opposite pole and finally surround the entire spindle by the time of the metaphase (Fig. 22). As the chromosomes begin to divide in the anaphase, there is no indication of any division of the mitochondria (Fig. 25). In the late anaphase, when the cell-constriction begins to appear (Fig. 27), the mitochondria begin to divide, so that when the cell-constriction is complete, the daughter cells (second spermatocytes) each contain approximately equal amounts of mitochondria (Fig. 28). The view of autonomous division is not supported here and it is quite evident that their division is due to their separation by the cell-constriction.

In the second maturation division, the behavior of the mitochondria is the same as in the first maturation division; they surround the spindle peripherally and become divided by the cell constriction (Fig. 29), so that the daughter cells (spermatids) receive equal amounts of mitochondria. As is usual in the insects, the mitochondria of the spermatid become resolved into a compact body, the Nebenkern (Fig. 30, *N*), which usually shows a lighter peripheral area. It is interesting to note that

There are many other cases in which the mitochondria are not localized at one pole of the nucleus in the spermatocyte, and while such localization is common, it is not general as Duesberg maintains.

in the giant spermatocytes (Fig. 59) the mitochondrial content is much greater than in the normal cells and the giant spermatids derived from such giant spermatocytes have Nebenkerns which are correspondingly larger than the normal ones (compare Figs. 30 and 31).

(*c*) *Transformation of the Spermatid.*—Besides the deeply staining Nebenkern, there are also present in the cytoplasm of the spermatids, an acrosome-sphere, a centrosome and often a chromatoid body (Fig. 30). The latter is found in some of the spermatogonia and some of the spermatocytes, passing undivided to one of the daughter cells at the time of mitosis. During the transformation of the spermatid, the chromatoid body is cast off in the elongating tail, as has been described by Wilson ('13) in *Pentatoma*.

The centrosome of the spermatid is derived from the centrosome of the second maturation division. In the late anaphase (Fig. 29), the centrosomes lie close to the chromatin masses at both poles and in the telophase, when the spermatid nucleus is reconstructed, it can still be seen closely adhering to the surface of the nuclear membrane (Fig. 30). The axial filament begins to grow from the centrosome and pierces the Nebenkern (Fig. 32). The division of the Nebenkern into two halves lying on each side of the axial filament is not quite so clear as in the case of other insects. In the further elongation of the spermatid (Fig. 33), the Nebenkern becomes drawn out, as the axial filament grows, into two narrow filaments forming a sheath around the axial filament.

What I have termed the "acrosome-sphere" is a derivative of the spindle; but whether it represents the "sphere" material or whether it is a portion of the mitosome could not be determined. It is a rather large compact body and stains intensely with hæmatoxylin. (Figs. 30, 32). As the spermatid goes through the stages of transformation, the acrosome-sphere becomes somewhat compressed and forms a typical acrosome at the head of the spermatozoön.

(*d*) *Discussion.*—Leaving aside for the present the question of the origin of the mitochondria, let us turn to consider some of the facts concerning the behavior of the mitochondria in spermato-

genesis. Not only do they have a characteristic behavior which seems to be quite general in the insects, but they are present in all generations of the male germ cells. There are many discrepancies in the literature on this point, some workers maintaining that the mitochondria disappear at various times. Buchner ('09) has described a disappearance of the mitochondria during mitotic division in the spermatogonia of *Gryllotalpa*, but on the contrary Duesberg ('10), working on the same form, has shown that the mitochondria do not disappear at that time. Wilke ('13) states that in *Hydrometra* the mitochondria are absent from some of the spermatogonia and in some cases from the spermatocytes. I am inclined to believe that the disappearances of mitochondrial structures is in all cases due to improperly fixed material. In fact, there is some evidence from Wilke's figures that this is the case. In the spermatocytes of *Hydrometra*, Wilke describes a deeply staining perinuclear zone of the cytoplasm in which are located "yolk-spherules" (Dotterkugeln). These spherules at first homogeneous, begin to show the appearance of threads within them and finally definite mitochondria are set free in the cytoplasm, being formed out of the substance of the "Dotterkugeln." His results, however, do not indicate that a reversal of this process might not be taking place. I have often seen in material which has been improperly fixed bodies resembling Wilke's "Dotterkugeln," which are produced by an artificial agglutination of the mitochondria. As I shall later attempt to show, certain methods of fixation may produce a variety of changes in mitochondrial structures ranging from a slight distortion of their form to their complete disappearance. I merely wish to urge the fact here that mitochondria are constant cytoplasmic structures of the cell and when proper technical methods are employed, they can be demonstrated at all periods in the cell-cycle.

The rôle of the mitochondria in spermatogenesis is difficult to interpret on the basis of their morphological behavior. The formation of the compact Nebenkern from the filar mitochondria may be an indication of some chemical change in the mitochondria or it may be merely an expression of the compactness which the other cell elements, notably the nucleus, show in the transforma-

tion of the spermatid into the spermatozoön. O. Vander Stricht calls the Nebenkern of the spermatid a "vitelline body." Wildman ('14) ascribes a nutritive function to the mitochondria (karyochondria) of the spermatid of *Ascaris*. The view has often been expressed that the mitochondria of the spermatozoön are concerned in locomotion, since they usually form a part of the tail. On the basis of the behavior of the mitochondria in spermatogenesis, we can only say that, just like the acrosome or the axial filament, they form a definite structural element of the spermatozoön, and it may be that their only significance is bound up with their function as an organelle of the spermatozoön. This view is contested by Meves, Duesberg and others on the basis of their observations on fertilization in certain forms where the mitochondria of the sperm apparently enter the egg and can be traced into the cleavage cells. If this behavior of the mitochondria can be found to be of general occurrence in fertilization, their significance from the point of view of heredity and development becomes of utmost importance. It may be said that the evidence for this is still in a very unsatisfactory state. I shall reserve for later discussion the questions bearing on this problem, until added observations on the mitochondria during oögenesis may be presented.

2. *Nutrition of the Egg.*

Without entering into a detailed description of the structure of the ovary of *Cicada*, I wish merely to mention some facts in regard to the nutrition of the egg during the growth period. The study of insect ovaries dates back over a century, many of the works being concerned with the question of the origin of the various cell-elements, viz., germ-cells, nurse-cells, epithelial cells and follicle-cells. Much of this work is of little value for the solution of these problems, since the study has been usually confined to that of the adult ovaries, and it is evident that only an embryological as well as a histological study of the ovary can enable us to come to any definite decisions. As pointed out by Hegner ('14), the origin of the nurse-cells and germ-cells may be different in different forms. In the case of *Miastor*, Hegner states that the nurse-cells are mesodermal in origin, while in the Chrysomelid beetles "the nurse-cells in the ovaries seem to be

of germ-cell origin" (Hegner, '14, page 119). In *Dytiscus*, *Giardina* ('01) has shown that germ-cells and nurse-cells arise by differential divisions of a stem-cell. In *Cicada*, I am unable to ascertain the origin of the cell-elements of the ovary from a study of the pupal and adult ovaries. The differentiation of the cells must take place at a comparatively early time in the long life cycle of the insect.

There are two ovaries, each consisting of a great many ovarian tubules, the ovaries of the adult being much larger than those of the pupæ, due to the presence of a large number of mature eggs. Figure 34 is that of a longitudinal section through an ovarian tubule of an adult recently emerged from the pupal case and it will at once be seen that it is a typical Hemipteran ovarian tubule. At the proximal end of the tubule is the narrow end-filament which is undoubtedly of a ligamentous nature helping to support the ovaries in the abdomen. The tubule may be divided into three zones depending on the character of the cells present. At the proximal end is the nurse chamber, containing all the nurse-cells. These stain very deeply and it is impossible to make out their cell walls. The chromatin of their nuclei is in the form of diffusely spread granules, and usually there is present a chromatic nucleolus and a true plasmosome (Fig. 45, *n.c.*). At the base of the nurse chamber (Keimlager) are found numerous young oöcytes in various stages of synapsis. Proceeding distally from the nurse chamber are the older oöcytes of the post-synaptic stages, those farthest away from the nurse chamber being the oldest and largest (Fig. 34, *oöct. 2*). In this region are also found the follicle-cells (*f.c.*) which begin to form definite follicles around the oöcytes. As the young oöcytes begin to migrate distally from the base of the nurse chamber, they still retain protoplasmic connections with the cytoplasm of the nurse-cells, resulting in the formation of pseudopod-like projections from the oöcytes, the egg-strings (*e.s.*), by means of which nutriment is passed from the nurse chamber to the oöcytes. Even old oöcytes in which yolk is beginning to form still retain connection with the nurse chamber by means of the egg-string (Fig. 47). In the ovaries of young pupæ the egg-strings appear simply as cytoplasmic protrusions of the oöcytes, but in the

adult ovaries, the egg-strings loose their cytoplasmic appearance and seem to be of a fibrous structure (Figs. 34, 45, 47, *e.s.*). This fibrous appearance of the egg-strings has often been figured in the ovaries of other insects, but I am inclined to doubt its normality; it seems rather that the fibrous appearance is due to fixation and really represents a compression of the cytoplasmic processes of the oöcytes.

In the nurse chamber, the nurse-cells are arranged in what appears to be a syncytium, and only occasionally can cell-walls be distinguished. In the young pupæ, the egg-strings of the oöcytes pass through the region of the follicle-cells into the nurse chamber and seem to fuse and become continuous with the cytoplasm of the nurse-cells. In the adult ovaries when all the cells are at the height of their functional activity, the egg-strings become much enlarged assuming the fibrous appearance above described. In the nurse chamber, the egg-strings end in a central fibrous mass (Figs. 34, 71, 72, *i.n.c.*), the substance of which is continuous with the egg-strings and from which they lead to the oöcytes. The nurse-cells immediately in the region of the central plasmatic mass stain deeply and show evidences of degeneration. Within the plasmatic mass can be seen many nurse-cell nuclei in various stages of disintegration (Figs. 72, 34, *i.n.c.*). The nuclei become smaller, staining intensely, and finally become broken down and the products of their disintegration can be seen passing down the egg-string into the oöcyte (Figs. 45, 71). The nurse-cells are thus ingested by the protoplasmic process of the oöcytes which are probably furnished with some substances (enzymes) enabling them to digest the nurse-cells. In the adult ovaries, at the height of the breeding season, the ingestion of the nurse-cells has taken place to such an extent that almost half of the nurse-cells have disappeared and the central plasmatic mass has grown in size containing a great many ingested nurse-cells. The growth of the central plasmatic mass is undoubtedly correlated with the disappearance of the nurse-cells in this region of the ovary.

It is a noteworthy fact that the ingestion of the nurse-cells takes place at a time when there is a rapid growth of the oöcytes. The oöcytes at the beginning of the synaptic processes have large

nuclei surrounded by a small amount of cytoplasm containing mitochondria. As the synaptic period progresses the oöcyte grows somewhat in size, chiefly by an increase in the size of the nucleus and a slight increase in the cytoplasmic volume. After the synaptic processes have been completed, the cytoplasmic volume increase rapidly, so that the oöcyte becomes over four times its linear size before yolk begins to form. After the cytoplasmic volume has reached its maximum, the yolk-building process commences and at the end of this period the almost mature oöcyte is about three times its linear size at the beginning of the process. This great increase in size is not due to any increase in cytoplasmic volume, but due to the great enlargement of the yolk spherules. It is doubtful whether the cytoplasmic volume increases at all after the beginning of the yolk formation.

The ingestion of the nurse-cells by the oöcytes in *Cicada* is perhaps homologous to the ingestion of the cells in the ovaries of other forms, the classical example of which is found in *Hydra*. As is well known, in *Hydra* one cell in the ovary grows large by ingesting the other cells in the ovary and becomes the functional oöcyte. In *Dinophilus*, Conklin ('06) has described the fusion of from 25 to 30 oögonia to form a single large (female-producing) egg. In *Ciona* and other Ascidians, the test-cells are ingested by the oöcytes and remain in the egg, perhaps aiding in the elaboration of nutrient materials, but are cast out of the egg prior to fertilization. In the ovaries of the certain insects (*e.g.*, *Dytiscus*) of the type in which the oöcytes are supplied with a separate group of nurse-cells, the process of absorption of the nurse-cells as the oöcyte grows has been often described. Korschelt ('86) has described in the ovaries of *Notonecta* and *Reduvius* a disintegration of nurse-cell nuclei in the nurse chamber through the action of the oöcytes, with the production of a central space, "Plasmatische Raum," in the nurse chamber, which is free from nuclei. Foot and Strobell ('11) have also described a similar plasmatic area free from nuclei in the ovaries of *Protenor*, but are not inclined strongly toward Korschelt's view that in this region the nurse cells disintegrate and furnish nutriment for the oöcytes. In *Cicada* a study of Figs. 34, 45, 71, 72 will show that there is no doubt that Korschelt's view is correct, for, not only

can nurse cells be found in various stages of disintegration, but the products of such disintegration can actually be seen to pass down the egg-strings to the oöcytes. From Fig. 45, the impression may be gathered that the material derived from the disintegrated nurse cells passing down the egg-string is accumulated in a zone about the nucleus. Such, however, is not the case and as will be shown later, the deeply staining perinuclear zone consists of mitochondria and in material fixed in Bouin's fluid, the mitochondria disappear while the granules in the egg-string are preserved.

3. *Mitochondria in Oögenesis.*

(*a*) *Mitochondria in Growth Stages.*—The young oöcytes of *Cicada* lying at the base of the nurse-chamber are at all times distinguishable from the nurse-cells not only by their characteristic nuclei, but also by the presence of definite aggregations of granular mitochondria which are characteristically localized in the cells.

During the entire synaptic period of the oöcyte, the mitochondria are found lying in the cytoplasm as a cap of deeply staining granules closely applied to the nuclear membrane at one pole (Plate V.). As will be seen from Figs. 38, 39, 40, the pole of the nucleus at which the mitochondria are found always corresponds to the pole of the nucleus towards which the synaptic threads are polarized. It will at once be seen by comparing Figs. 17 and 40, that the mitochondria of the oöcyte and spermatocyte are localized in the cytoplasm at homologous positions, namely at the side of the nucleus where the idiozome, the sphere or centrosome is probably located. In the oöcytes, however, no centrosome or sphere can be distinguished and similarly Montgomery ('11) states that in *Euschistus* "it is not clear whether this body (idiozome or sphere) has any homologue in the oöcytes," etc.

The mitochondria of the oöcytes of *Cicada* are always in the form of granules, never assuming the filar form as found in the spermatocytes. During the later synaptic stages (pachytene stage, Fig. 40) there is an increase in the amount of mitochondria, but their localization at one pole remains constant. In the post-synaptic stages we find that, although the mass of mito-

chondria is still found at one pole of the nucleus, they gradually completely encircle the nucleus. After this period, when the synaptic threads have become diffuse and the "germinal vesicle" is established, the mitochondria increase greatly in numbers and are found localized in a zone immediately surrounding the nucleus (text-fig. 3, and Figs. 83, 84).

This perinuclear zone of mitochondria is sharply delimited from the rest of the cytoplasm. Fauré-Frèmiet ('08) has described the mitochondria of the oöcytes of *Julus* as being similarly arranged in a perinuclear zone whose cytoplasmic limit is definitely marked by the presence of a membrane formation. I can find no evidence for the presence of such a limiting membrane in *Cicada*. The mitochondria continue to increase greatly in numbers in the perinuclear region up until the stage in which the cytoplasmic volume of the oöcyte is greatest (Fig. 85). Throughout the period in which mitochondria are forming in the perinuclear zone, the nuclear membrane remains intact at all times as is evidenced in material improperly fixed in which the nuclear membrane has shrunken away from the cytoplasm as shown in Fig. 85. Neither is there any evidence that any nuclear materials are discharged or extruded into the cytoplasm at any time. There are many descriptions in the literature of bodies in the cytoplasm of oöcytes which have been derived from the discharge of nuclear materials. Goldschmidt and his pupils have maintained that the mitochondria are derived from the passage of nuclear materials into the cytoplasm, but there is no evidence that this is the case in *Cicada*. Vejdovskỳ ('12) has described in the oöcytes of *Aphrophora* large deeply staining globules and vacuoles (compare my Fig. 42). He does not relate these structures to the mitochondria in any way, but indicates that these nucleolar-like bodies in the cytoplasm are derived by a "nucleolization" of the chromosomes and a casting-out of the resulting nucleoli into the cytoplasm. He is of the opinion that the vacuoles represent the escaped nuclear sap, there being no nuclear membrane present at this time. In the first place, it seems very doubtful that the nuclear membrane does disappear at any time in the resting nucleus. Secondly, if there is a nuclear membrane present it is difficult to see how such solid bodies as nucleoli

could pass through the membrane unless they be in a fluid, diffusible state, and if so they would become diffused when they entered the cytoplasm. Cases of "extruded nucleoli" have often been mentioned in the literature, but it must be said that many of the interpretations are exceedingly doubtful. In *Cicada*, when the ovaries have been fixed for a considerable length of time (24 hours) in Bouin's fluid, the mitochondria entirely disappear, leaving occasionally traces of their dissolution in the form of cytoplasmic vacuoles in the region of the nucleus (Fig. 82). By fixing the ovaries in Bouin's fluid for varying lengths of time (5 to 12 hours) a variety of peculiar structures may be found in the cytoplasm which are all referrable to the various stages and degrees of dissolution of the perinuclear zone of mitochondria (Figs. 39, 41, 42, 80, 81, 82). When the ovaries are fixed for ten hours in Bouin's fluid, a zone of the cytoplasm is found around the nuclei of the oöcytes which takes the plasma stain intensely (Fig. 42). Within it may be found vacuoles and deeply staining bodies resembling those structures which Vejdovsky ('12) figures in *Aphrophora*. In studying the effect of acetic acid of fixing fluids upon the mitochondria, I am led to believe that many of the peculiar cytoplasmic bodies which have been described and figured in germ-cells under various names such as extruded nucleoli or plasmosomes, vacuoles, idiozomes, spheres, yolk-spherules (Dotterkugeln), etc., are the result of imperfect fixation of mitochondrial substances. O. Vander Stricht ('04) has shown a similar effect of reagents in the distortion and dissolution of mitochondria in the "couche vitellogène" of the oöcyte of the bat.

(*b*) *Yolk-formation.*—Throughout the growth period of the oöcyte, the mitochondria increase greatly in numbers and continue to be located in the well delimited perinuclear zone. At the time when the cytoplasmic volume of the oöcyte is at its maximum (Fig. 85) the zone of the mitochondria occupies approximately a third of the cytoplasmic volume. After this stage in the growth of the oöcyte, the perinuclear mitochondrial mass begins to loose its well-defined zonal limits and the granules become dispersed in the cytoplasm towards the periphery of the oöcyte (Fig. 86). The migration of the mitochondria from the

perinuclear zone to the periphery of the cell continues, until in the older oöcytes all the mitochondria have been localized in the cortical region of the oöcyte (Fig. 47). A similar centrifugal migration of the mitochondria from the perinuclear zone toward the cell periphery has been described by Payne ('16) in the oöcytes of *Gryllotalpa*, by Fauré-Frèmiet ('08) in the oöcytes of *Julus*, by Govaerts ('13) in the oöcytes of the beetles *Trichiosoma* and *Cicindella*, and by many other workers who have studied the mitochondria in the oöcytes.

At this time the cytoplasm of the oöcyte is of a hyaline, homogeneous appearance, except for the mitochondrial granules located in the peripheral zone. In somewhat older oöcytes (in which yolk-spherules have not as yet formed) numerous vacuoles appear in the cortical zone of the cytoplasm where the mitochondria are located. These vacuoles are at first very small in size and within them can be seen the mitochondrial granules. That the granules in the vacuoles are actually mitochondria is proved by the fact that they respond to all the specific stains and are only found in material which has been fixed according to the mitochondrial technique. The further history of the mitochondria and the vacuoles of the cortical layer is concerned with the process of formation of the yolk-spherules. The mitochondria within the vacuoles begin to disintegrate, often showing small vacuoles within themselves. At this time the vacuoles containing the disintegrated mitochondria appear very much like numerous small nuclei. These structures are no doubt similar to the "pseudo-nuclei" of Blochmann. Korschelt ('89) has described similar "pseudo-nuclei" in the oöcytes of several insects, and he considers them as follicle-cell nuclei which have migrated into the oöcyte much as the test-cells of the Ascidians migrate into the oöcyte to aid in the elaboration of nutrient materials. Hegner ('15) has studied similar "secondary nuclei" in the oöcytes of *Camponotus* (Hymenoptera) and has also given a satisfactory review of the literature dealing with these structures. However, he is of the opinion that the "secondary nuclei" arise as buddings from the oöcyte nucleus and that this process is, hence, comparable to chromatin diminution processes in other forms (*e.g.*, *Miastor*, *Ascaris*). Hegner finds that the "secondary

nuclei" surround the oöcyte nucleus increasing in numbers presumably at the expense of the oöcyte nucleus. Later they become scattered toward the periphery of the oöcyte, increasing in numbers and finally disappearing. According to Blochmann the "pseudo-nuclei" may become incorporated in the yolk-spherules. From this general behavior of the "pseudo-nuclei" and the "secondary nuclei" of Hegner I am convinced that in all cases they represent stages in the transformation of mitochondria into yolk. Loyez ('08) has shown that these structures are not nuclei in any sense of the word and similar to Govaerts ('13), she has shown their relation to mitochondria and yolk-formation.

Figure 48 shows the various stages in the development of the yolk-spherules from the substance of the mitochondrial granules contained in the vacuoles. The substance of the vacuoles takes the plasma stain lightly at first, and as the vacuole grows in size the mitochondrial granules disappear and the substance of the vacuole begins to take the plasma stain more deeply. These plasma staining bodies grow considerably in size and often a small area of their substance begins to show a marked affinity for the basic stains. This basic staining area grows in extent and finally the entire body, which is none other than a yolk-spherule, takes the basic stain intensely. The relative size of the yolk-spherules is enormous as compared to the size of the mitochondrial granules from which they have been derived, and this indicates, together with the changes in the staining reactions, that a series of chemical reactions take place during this transformation. The mature egg is filled with these large deeply staining yolk-spherules, between which lie small granules which give the characteristic mitochondrial staining reactions similar to the mitochondria described by Duesberg ('08) in the egg of the bee. Apparently not all of the mitochondria are transformed into yolk; or perhaps new ones are being formed which have as yet not been transformed into yolk.

The transformation of mitochondria into yolk has been described by Loyez, Russo, Fauré-Frèmiet, Govaerts, Hegner, Lams, Vanderstricht, and others. L. and R. Zoja have described a transformation of the "plastidules fuchsinophiles" into yolk

in the egg of *Helix*. R. Vander Stricht ('11) has described in the oöcyte of the cat a "couche vitellogène" or perinuclear ring of mitochondria which grows in size during the early growth stages of the oöcyte and which later becomes dispersed toward the periphery of the cytoplasm and there gives rise to yolk. O. Vander Stricht ('94) has also described a "couche vitellogène" (of mitochondrial nature) in the oöcytes of the bat, which in the young oöcytes is arranged in the perinuclear zone, but in the older oöcytes it becomes diffused in the cytoplasm. This diffused substance forms the "pseudochromosomes" of the oöcytes of the adult ovaries and from these are derived the yolk bodies. Wildman ('13) has described two kinds of cytoplasmic inclusions in the spermatocytes of *Ascaris*, the "karyochondria" and the "plastochondria," both of which are derived from nuclear material. The "karyochondria" become transformed into yolk granules which later fuse to form the "refractive" body of the spermatid. The "plastochondria" have a negative behavior and take no part in spermiogenesis. Recently Gajewska ('19) has described a perinuclear ring of mitochondria in the oöcytes of *Triton* and indicates the relation of this to yolk formation.

I have cited only a few of the cases in which the mitochondria appear to have a genetic relationship to yolk formation, and as will be shown later, this relation is more general than is usually supposed. The chemical processes involved in the transformation of mitochondria into yolk cannot involve complicated chemical changes since the mitochondria themselves are of a phospholipoid nature and closely allied chemically to yolk.

(*c*) *Discussion*.—In almost every work dealing with oögenesis and the study of oöcytes, mention is made of certain cytoplasmic inclusions which are either of a granular, globular or filar nature, or else assume larger proportions and appear as single compact bodies. Various names have been applied to these structures, such as "Dotterkern" or yolk-nucleus, yolk-matrix, "couche vitellogène," "corps de Balbiani," pseudochromosomes, extruded nucleoli, "zona plasmatica perinucleare," etc. In nearly all these cases it will be noted that these special portions of the cytoplasm are involved in the process of yolk elaboration, and I shall attempt to point out some of the homologies existing be-

tween the above-named structures and the perinuclear zone of mitochondria as found in the oöcytes of *Cicada*. First, however, it may be said that the occurrence of a special zone of mitochondria immediately surrounding the nucleus is by no means uncommon. Fauré-Frèmiet ('08) has described a perinuclear ring of mitochondria in the oöcytes of *Julus*, as does Payne ('77) in the oöcytes of *Gryllotalpa*, Vejdovský ('12) in the spermatocytes of *Diestramena*, Schaefer ('07) in the spermatocytes of *Dytiscus*, Shaffer ('17) in the spermatocytes of *Passalus*, etc. In fact in almost every case where the mitochondria have been studied in the early growth period of the germ-cells, they have been found in close spatial relations to the nucleus.

In the early literature dealing with insect oögenesis, there is almost always figured granules or deeply staining areas of the cytoplasm whose homology with the perinuclear ring of mitochondria as described here in *Cicada* becomes at once evident. Korschelt ('89) describes in the oöcytes of *Dytiscus* deeply staining portions of the cytoplasm in the region of the nucleus which he interprets as representing nutrient materials derived from the nurse-cells, and into which the nucleus of the oöcyte sends amœboid processes. Marshall ('07*a*) describes small nuclear-like bodies around the nuclei of the oöcytes of *Polistes*. These increase in number and later migrate to the periphery of the oöcyte, but Marshall offers no explanation as to their significance. The same author (Marshall, '07*b*) figures a deeply staining granular zone around the nucleus of the oöcyte of *Platyphlax* (Hymenoptera). Often this granular mass is cone-shaped extending toward the egg-string (compare my Fig. 45). In the older oöcytes, he describes the appearance of deeply staining bodies which lie scattered in the cytoplasm (when fixed in Flemming's fluid); but their further history is not traced. McGill ('06) describes a deeply staining perinuclear zone of granules in the oöcytes of *Plathemis* and *Anax*, which she considers as the "yolk-nucleus." It later breaks away from the nuclear wall and becomes scattered in the cytoplasm. Hegner ('15) describes a perinuclear zone of granules in the oöcytes of *Camponotus* which he states "resembles chromatin in some respects and may represent chromatin which has passed through the nuclear membrane

into the cytoplasm" (page 508). More recently, Nakahara ('18) has described in the oöcytes of *Perla* a deeply staining mass of granules in the cytoplasm closely surrounding the nuclear membrane, which he calls the "yolk-nucleus." This later breaks away from the nuclear membrane, becomes insignificant and finally disappears. Nakahara is of the opinion that the nucleoli of the oöcyte nucleus are derived from the passage of material from the "yolk-nucleus" into the oöcyte nucleus, and hence nucleoli are extra-nuclear in origin. These interpretations of Nakahara are hardly excusable since at this late date he has had the advantage of a large mass of data bearing on mitochondria, and it is evident that he has not made a proper study of the literature. Many similar cases of almost riotous interpretations of mitochondrial structures might be mentioned, many of them inexcusable in light of the recent studies on the mitochondria. As has been before stated, many of these erroneous interpretations are based upon material prepared without regard for the special technical processes involved for the demonstration of mitochondrial structures. Perhaps the best example of this is the work of Giardina ('04). This writer has studied the oöcytes of a number of insects (*Mantis*, *Periplaneta*, *Stenobothrus*, *Gryllus*) and the gastropod, *Helix arvensis*. In all of these forms, Giardina has described a special zone of the cytoplasm immediately surrounding the nucleus to which he assigns the name "zona plasmatica perinucleare." At times this zone may appear granular, striated, vacuolated, homogeneous, etc., and is always sharply delimited from the rest of the cytoplasm by a membrane. After considerable discussion as to its physical state, Giardina comes to the conclusion that it is a formation *in situ* of the cytoplasm under the action of substances from the nucleus, and that it acts as an intermediary between the nucleus and the cytoplasm in the nutrition of the egg. From a study of Giardina's figures and a comparison with the oöcytes of *Cicada* fixed in Bouin's fluid for varying lengths of time (Figs. 39, 41, 42), it becomes at once evident that the "zona plasmatica perinucleare" of Giardina is nothing more than poorly-fixed mitochondria arranged in a perinuclear zone. Duesberg ('12) is of this opinion as is also Fauré-Frèmiet ('10).

It thus seems that perhaps it is quite general in the insects that the mitochondria of the oöcyte are first arranged in a definite zone around the nucleus during the period in which they increase in numbers, and in the older oöcytes the perinuclear zone of mitochondria becomes dispersed towards the periphery of the oöcyte and becomes concerned in the process of yolk-formation.

4. *Significance of the Perinuclear Zone of Mitochondria.*

(*a*) *Relation to Yolk-nuclei.*—As I have before indicated, the occurrence of a perinuclear zone of mitochondria is quite commonly met with especially in the female germ-cells during the growth period. I shall not attempt a lengthy review of the literature in this regard since we already have the excellent review of Duesberg ('12), but I wish to point out some of the significant facts which have a bearing on the problems presented here. I wish merely to discuss the origin and homologies of the yolk-nucleus or "corps de Balbiani," mention of which has been made in almost every work in oögenesis which has appeared in the past two decades. As has been shown in Duesberg's ('12) review, much of the recent work in this connection has demonstrated that these bodies (yolk-nuclei) are in many cases partly constituted of mitochondrial substance. O. Vander Stricht ('04) has shown that the "couche vitellogène" surrounding the nucleus of the oöcyte of the bat is of a mitochondrial nature, and von Winiwarter and Sainmont ('09) have described a zone of granular mitochondria around the nucleus of the oöcyte in the kitten, which they homologize with the yolk-nucleus and "couche vitellogène" of Vander Stricht. Recently, Gajewska ('19) has shown that in *Triton* the yolk-nucleus is made up of three substances: "Zuerst eine Anhäufung von Ergastoplasmatischer Substanz (Ergastoplasma-Dotterkern), dann ein Mitochondrienkonglomerat (Dotterkern als Körnerkonglomerat) und endlich ein Haufen von Fettkugeln und Eiweissplättchen. Die Muttersubstanz fur den Dotterkern ist der perinukleare Ring (couche vitellogène)" (p. 116).

The fact which I wish to emphasize here is that in all cases the yolk-nucleus has its beginning in the young oöcyte in the form

of granular masses closely applied to the nuclear membrane, and these masses grow in amount during the period in which they are close to the nucleus. This is clearly shown in the early works on the yolk-nucleus such as Munson ('99) in *Limulus*, Crampton ('99) in *Molgula*, Calkins ('95) in *Lumbricus*, Van Bambeke ('95) in *Scorpæna scrofa*, Vander Stricht ('98) in the human oöcytes and in *Tegnaria*, and by others of the early workers on these structures. In almost all these cases, the close association of these granules with the nucleus and the similarities in the staining reactions with that of the chromatin have given the earlier workers the opinion that the granular masses represent extruded nuclear material. In many of these cases, the granular masses surround the centrosome forming a compact body with sharp outlines.

The formation of the typical yolk-nucleus (corps de Balbiani) as found in the Arachnids has been described by Vander Stricht ('98, '04) and Faurè-Frèmiet ('10). Here, as in other cases, the granules (mitochondria) forming the yolk-nucleus are first found in a perinuclear zone. Within this granular mass is found a deeply-staining vesicle, and later the mitochondria gather about it in concentric layers forming the typical compact Arachnid yolk-nucleus, which usually shows a ray-like structure. The relation between the yolk-nucleus and the attraction-sphere has often been noted. I have previously called attention to the fact that the mitochondria are usually (although not always) localized at the pole of the cell at which the centrosome, sphere or idiozome lies. In the formation of the compact Arachnid yolk-nucleus, the mitochondria gather about the centrosome-sphere as a center, the relation between the two being only spatial. Duesberg ('12) has noted this relation and applies the term "idiozom" or "centrotheka" to the central vesicle of the yolk-nucleus and points to the fact that this conception corresponds to Henneguy's definition of the "corps de Balbiani": "Un corpuscle centrale entouré d'un zone d'aspect homogène ou finement granuleux."

It thus seems that in oöcytes the perinuclear ring of mitochondria may behave in two ways; it may become dispersed in the cytoplasm and give rise to yolk in the periphery of the cell (as seems to be the case in the insects), or the mitochondria of the

perinuclear zone may become massed about the centrosome, forming the compact "corps de Balbiani" which later disintegrates in the formation of yolk (as in the Arachnids, etc.).

(*b*) *Origin of the Mitochondria.*—There are two facts of importance to be noted in the foregoing discussion: (1) the quite usual presence of mitochondria in a zone of the cytoplasm immediately surrounding the nucleus; (2) the presence of this perinuclear zone at a time when the mitochondria are increasing greatly in number. One of the most important questions bearing on the nature and rôle of the mitochondria is bound up with the mode of their origin and increase in number, and there has been considerable controversy regarding these questions. According to the view of Meves, Bouin, Duesberg and others, the mitochondria have no "de novo" origin, but are always derived from pre-existing mitochondria by a process of division. The chief evidences to support this view are: (1) the constant presence of mitochondria in all cells at all times; (2) the mitochondria within the cell are actually distributed to the daughter cells at the time of mitosis; (3) in some few cases (Meves in *Ascaris*, Duesberg in *Ciona* and *Apis*) the mitochondria of the fertilized egg have been traced into the embryonic cells. In the first place, it may be said that the omnipresence of mitochondria in all living cells may be interpreted upon an entirely different basis as I shall attempt to show later. There is, of course, a genetic continuity of mitochondria to a certain degree; the mitochondria of a cell are certainly carried into the daughter cells at the time of mitosis, but the view that individual mitochondria give rise to "homologous" mitochondria of succeeding cell-generations is entirely without evidence. Duesberg ('18) found that the yellow oöplasm of the Ascidian egg was rich in mitochondria, and, using the results of Conklin ('05) who traced yellow oöplasm of the egg into the embryo, consequently maintained that the mitochondria were genetically continuous from the fertilized egg to the embryonic cells. While there is no question that the yellow oöplasm is continuous, yet this is far from establishing that the individual mitochondria are continuous.

Opposed to the "genetic continuity" hypothesis of the mitochondria, we have the "chromidial" hypothesis developed by

Goldschmidt ('09) and his students. As has been before mentioned, there have been many descriptions of cytoplasmic bodies whose origin has been attributed to material extruded from the nucleus. According to Goldschmidt and others (Buchner, Jorgensen, Schaxal, Wasilieff, Popoff, etc.), mitochondria are derived from the chromidia and ultimately from the chromatin of the nucleus, and are hence similar to Hertwig's "chromidia." The fact that the mitochondria lie at the pole of the nucleus where the idiozome lies and toward which the synaptic threads are polarized, has been taken by Buchner and Wasilieff to be the place where mitochondria arise by the emigration of chromatic materials from the nucleus into the cytoplasm. Buchner ('09) derives the mitochondria of the spermatocytes of *Gryllus* from the material of the sex-chromosome, which in the bouquet stage becomes vacuolated and shows evidences of disintegration. While chromatin from the nucleus may at times come to lie in the cytoplasm (*e.g.*, chromatin diminution processes in *Ascaris* and *Miastor*, etc.), yet there is no strong evidence that it may give rise to the mitochondria. It is difficult to see how escape of chromatin from the nucleus could account for the tremendous increase in the mitochondria as found during the growth period of the oöcytes of *Cicada*. Furthermore, the difference of behavior of chromatin and mitochondria towards fixing fluids and specific stains indicates that they are of a totally different chemical nature (see Cowdry '16, p. 426).

According to the view of Vejovskỳ ('07, '12) the mitochondria are cytoplasmic structures having their origin in the "regressive modification" (Duesberg, '12) of the sphere material. Montgomery ('11) expresses the opinion that "it is probable that they (mitochondria) are produced by either idiozome or nucleus or by a joint action of both" (p. 787). Although it is quite usual that the mitochondria are found lying close to the idiozome or sphere, there is no conclusive evidence that they have their origin in or from the sphere material.

We have now discussed the three views prevalent regarding the origin of the mitochondria. From a study of the mitochondria of *Cicada* and from a review of much of the literature bearing on the subject, still another view presents itself which

has hitherto been only occasionally expressed in the literature. This is what might be called the "interaction theory," and according to it the mitochondria are neither self-perpetuating structures, nor are they derived from the chromatin of the nucleus, nor by a disintegration of the sphere material. According to this view the mitochondria are cytoplasmic differentiations which arise through specific chemical actions of the nucleus upon materials in the cytoplasm. I have before emphasized the almost universal presence of a particular zone of mitochondria immediately surrounding the nucleus at certain times in the cell-cycle, and I believe this perinuclear zone to be one of the best morphological demonstrations of an "interaction" between nucleus and cytoplasm. The presence of this perinuclear zone of mitochondria during the period in the oöcyte when the mitochondria are increasing in number as the cytoplasmic volume is also growing by assimilation of products from the nurse-cells, supports the view that the mitochondria are being built up by nuclear action on substances in the cytoplasm. After the maximum amount of mitochondria has been elaborated, the perinuclear zone is dissipated and the mitochondria become diffusely spread in the cytoplasm followed by their transformation into yolk.

Montgomery ('11) and Browne ('13) have also expressed views that the mitochondria may arise as a result of the interaction between nucleus and cytoplasm.

The view of mitochondrial continuity has no more than weak circumstantial evidence to support. Granting that the mitochondria of the spermatozoa are brought into the egg and that the cells of the embryo and adult all possess mitochondria, it is far from establishing the fact that the mitochondria of these cells are derivable from those of previous cell-generations which go back to the fertilized egg. Mitochondria do arise, as such, *de novo* in the cells by the chemical actions of the nucleus on the cytoplasm immediately surrounding it, although there may be a limited amount of mitochondria carried over from the previous cell-divisions.

Just what portion of the cytoplasm is acted upon by the nucleus is another matter. In the oöcytes of *Cicada*, nutriment is

brought in from the nurse chamber through the egg-string and assimilated by the cytoplasm; the nucleus exerts a chemical influence (enzyme?) on these products whereby the mitochondria are differentiated in the cytoplasm immediately surrounding the nucleus. According to this view it is possible to explain the presence of mitochondria in all cells (animal and plant) at all times, for all cells are constantly receiving nutriment which is taken into the cytoplasm (assimilated) and then acted upon by the nucleus resulting, among other things, in the elaboration of mitochondria. It thus seems that the *mitochondria arise as differentiated parts of the cytoplasm through specific chemical (enzyme) reactions of the nucleus upon the products of assimilation of the cell.* What the significance of such cell structures in the cell economy may be is quite another problem.

E. General Considerations.

1. *Chromosomes.*

My study of the chromosomes of *Cicada*, I believe, presents added evidence to the already large body of facts bearing on the individuality of the chromosomes and my observations indicate a persisting chromosomal organization which is constant throughout the cell-cycle. I have studied the metaphase plates of hundreds of cells, germinal and somatic, and have found no variations in either chromosome number, the relative sizes of the chromosomes or their characteristic grouping. The only exceptions to this is found in the giant spermatocytes and the multinuclear cells in the adhesive gland of the female, where the increase in chromosome number is due to the suppression of the division of the cell-body at mitosis resulting in the formation of polyvalent chromosome groups. In such polyvalent cells, we can still recognize double, triple, quadruple, etc., sets of each of the chromosome pairs.

There have appeared at various times in the cytological literature discrepancies of chromosome numbers in certain species, purporting to show that chromosomes vary in number and cannot be regarded as persistent structures of the cell. McClung ('17) has dealt ably and at length with such criticisms and has particularly concerned himself with the work of Delle Valle. Ac-

cording to Delle Valle, chromosomes are not constant structures of the cell and their number varies as ordinary fluctuating variations, since the number in a particular cell depends upon the mean size of the chromosomes, the amount of chromatin in the nucleus being constant (McClung, '17, p. 548). I have already (page 409) called attention to the remarkable difference between the metaphase chromosomes of the young follicle-cells and those of old follicle-cells (compare Figs. 7, 10, 11). In the old follicle-cells, the chromosomes are somewhat longer, thinner and poor in chromatin constitution. The nuclei from which such chromosomes are derived are much poorer in basichromatin than the nuclei of the young follicle-cells, usually having only a single small mass of basichromatin. Nevertheless, as will be seen from Figs. 10 and 11, the chromosome number remains constant and their relative sizes are similar to the chromosomes of other diploid groups; their only difference seems to be that they possess less chromatin. It is at once evident that this condition cannot be interpreted on the basis of Delle Valle's hypothesis. It also brings to light the fact that the material concerned in maintaining the chromosome number and size is not the chromatin of the nucleus, which is apparently more or less variable in amount, but the underlying structural basis of the chromosomes, namely the linin. In a previous paper (Shaffer, '20), I have emphasized the importance of the linin as being responsible for the architecture and organization of the chromosomes and for the maintenance of the stability of the nuclear elements. The chromomeres of the chromosomes are linearly arranged in a definite order (Wenrich, '16) which is maintained constant through the agency of the linin, the structural basis. Besides this, the linin is also concerned in the movements and localization of the chromatic elements of the nucleus. Some of the most fundamental problems of the cell are bound up with such phenomena, namely, what determines how the chromomeres shall be arranged in the chromosome, or by what agency are homologous chromosomes brought together in synapsis. "As long as 'conjugation' of the chromosomes is dealt with as though they were entities with independent power of movement, instead of the processes back of it, the super-

structure of theory must remain as unwieldy as at present" (Kingsbury, '12, p. 48). As I have before pointed out, it is possible that we may find an explanation for such movements of the chromatic elements in the linin ground-work of the nucleus. Homologous chromosomes have linin connectives running between them and it is possible that their union in synapsis is brought about by a contractility of these interchromosomal linin fibers, very much as the spongioplasm acts in localizing substances in the egg (Conklin, '17). The linin is, therefore, the persistent material of the nucleus, while the chromatin may be variable in amount in certain phases of the cell-cycle.

(*b*) *Mitochondria.*—The function or the rôle of the mitochondria in the cells of animals and plants still remains one of the unanswered cytological problems of to-day. A few cytologists have insisted that the mitochondria are idioplasmic materials which have a rôle in the transmission of hereditary characters similar to the chromosomes. According to this view, the chromosomes bear the determiners for the generic or racial characters of the organism, while the mitochondria bear the determiners for the specific or individual hereditary characters. From what little we do know of "cytoplasmic inheritance" it seems that the reverse is true (Conklin, '17) and that the larger orientations of development are fixed by the cytoplasm, particularly that of the egg. The idioplasmic view of the mitochondria was developed from the fact that they were found in all cells at all times and that they behaved characteristically during mitosis, becoming equally distributed to the daughter cells. These facts seemed to indicate a persistence and continuity of the mitochondrial substance through the cell-cycle which would fit in with the view of their idioplasmic nature. As I have before indicated (p. 445) there is no basis for maintaining their genetic continuity, but rather that "new" mitochondria may be formed in the cell without any relation to previously existing mitochondria.

According to another view the mitochondria may become transformed into certain histological elements of the cell, such as muscle and nerve fibrillæ, collagenic fibrils, and certain of the glandular secretions (pancreas, thyroid, etc.). Without

entering into a detailed discussion of the "histogenetic" view of the mitochondria, it may be said that the evidence is far from being convincing. From what we know of the chemical nature of the mitochondria, it becomes difficult to understand how they may become transformed into structures so different chemically. According to Cowdry ('16, p. 435), "it is apparent that the doctrine of an actual chemical transformation of mitochondria into substances of diverse constitution is weak."

As to the chemical nature of the mitochondria, practically all workers agree that they are combinations of lipins with varying amounts of albumin (phospholipins). The transformation of the mitochondria into the yolk-spherules of the egg at once indicates their lecithin nature. N. H. Cowdry ('17) has given a summary of the more important data bearing upon the chemical nature of the mitochondria. Löwschin ('13) has been able to make mitochondria artificially in lecithin and albumin solutions. These mitochondria behave in every way (form, solubility, fixation and staining) like true mitochondria of organic cells. Russo ('12) has described an increase in the number of mitochondria of the oöcytes of the fowl following injections of solutions of lecithin.

While we are beginning to know something about the chemical nature of the mitochondria, we are far from knowing their rôle in the physiology of the cell. That they bear an important relation to metabolism is conceded by many workers, and the class of chemical compounds to which the mitochondria are allied chemically (the lipins) have recently been emphasized in biochemical works as being intimately concerned in metabolic processes. In fact, Mathews ('15) believes that the phospholipins are the most important substances in organic matter.

In the oöcytes of *Cicada* it is quite clear that the mitochondria are related to the nutritive metabolic processes and that they are actually transformed portions of the products of assimilation. According to Cowdry ('17), in plants the "mitochondria are concerned in the formation of chlorophyll, and thus the very existence of the plant depends upon them." Maclean ('18) fed one group of hens on a normal diet and another group on a diet free from fats and lipins and concluded that the "lipins play an

important, if as yet unknown part in the history of fat metabolism" (p. 172).

Some workers (Kingsbury, '12, Cowdry, '17, etc.) have ex-expressed the view that the mitochondria are concerned in the processes of cell-respiration, but there has not been sufficient experimental work to support this view. Maclean ('18) points out some very interesting relations between lipins and oxydative processes and mentions the work of Stanewitch who found that wheat embryos treated with solvents which extracted most of the lipins showed a respiration energy which was lower than normal.

It would, I think, be premature to make any definite statement as to the function or significance of the mitochondria in organisms until we know more of the biological significance of the lipins. Maclean says (p. 107):

"From what has already been said regarding the unsatisfactory state of our knowledge of the lipins, it follows that their exact function in the animal and vegetable economy is necessarily obscure. Their great importance is proved by their general occurrence in every cell, but little or no direct experimental proof indicating their specific function has yet been obtained. When we consider the obscurity in which the chemistry of the lipins has been shrouded and the fact that even now, in many cases, is not satisfactorily established, it is easy to understand that many of the properties and functions ascribed to these bodies are based on little more than the imagination."

For the present, all we can say regarding the function of the mitochondria is that they form the major part of the lipoid constitution of organic cells, and when we know more of the biological significance of lipins, we shall know more concerning the rôle of the mitochondria in cell economy. Obviously, further progress in this direction must come along experimental lines.

F. Summary.

1. The chromosome number in all male diploid groups of *Cicada* (*Tibicen*) *septemdecim* is 19, and in the female diploid groups 20.

2. The diploid chromosome groups are characterized by the

presence of one large pair of chromosomes (the macrochromosome pair, *AA*) and two pairs of somewhat smaller chromosomes (*BB*, *CC*). The other 13 chromosomes show no size differences.

3. There is no variation in chromosome number or in their form and arrangement in any of the diploid groups studied.

4. In the spermatocytes there are two ring tetrads of the *Stenobothrus* type, which are derived from the *AA* and *BB* chromosome pairs of the spermatogonia. These tetrads divide reductionally in the first maturation division.

5. An odd chromosome is present which persists as a nucleolus in the growth stages of the spermatocyte. It passes undivided to one pole in the first maturation division and divides in the second division.

6. The tetrads are always grouped characteristically in the metaphase of the first maturation division and the same grouping is found in the metaphase of the second spermatocytes.

7. The synaptic stages in the oöcyte were studied, giving evidence that the chromosomes pair side-to-side (parasynapsis).

8. Two chromatin nucleoli are present in the preleptotene stages of the oöcyte. These disappear in the synaptic stages and reappear in the post-synaptic stages. These nucleoli are interpreted to represent the two sex-chromosomes of the female which go through a synaptic phase like the autosomes.

9. Mitochondria are found in the spermatogonia in the form of granules localized at the end of the cell bordering on the cyst cavity.

10. The first evidences of a degeneration of a spermatogonium is shown by an agglutination of the mitochondria. As degeneration continues the mitochondria continue to agglutinate forming large lipoid globules in the cell, evidently yielding a fatty degeneration.

11. In the spermatocytes the mitochondria are filar; they surround the spindle peripherally at the time of the maturation divisions and become divided by the cell-constriction. The mitochondria of the spermatid form the round compact Nebenkern which later becomes drawn out as a sheath surrounding the axial filament of the spermatozoon.

12. The ovaries of *Cicada* are typically Hemipteran in struc-

ture. The egg-strings of the oöcytes pass up into the nurse chamber and serve to carry the nutrient materials to the oöcytes. In the ovaries of the adult, the nurse-cells are ingested at the upper end of the egg-string and their ingested products pass down into the oöcyte as nutrient materials.

13. Mitochondria are found in the young oöcytes as a deeply staining mass of granules lying in the cytoplasm at one pole of the nucleus. In the later stages the mitochondria gradually extend around the nucleus forming a perinuclear zone of mitochondria.

14. The mitochondria increase greatly in numbers during the postsynaptic stages still retaining their perinuclear arrangement.

15. When the cytoplasmic volume of the oöcyte has reached its maximum, the perinuclear arrangement of the mitochondria becomes lost and the mitochondria become dispersed toward the periphery of the oöcyte.

16. At the periphery of the oöcyte, the mitochondria become transformed into yolk-spherules. First vacuoles are found surrounding the mitochondria, these structures resembling the "pseudo-nuclei" of Blochmann. The substance of the vacuoles at first takes the plasma stain lightly and as it grows in size, it becomes more and more deeply staining. The globules increase greatly in size and show a marked affinity for the basic stains.

17. The relation between the perinuclear zone of mitochondria and such structures as yolk-nuclei, etc., is pointed out.

18. The zone of the cytoplasm immediately surrounding the nucleus is taken to be the locus in the cell of the formation of mitochondria through the chemical action of the nucleus upon the products of assimilation taken in by the cytoplasm.

LITERATURE CITED.

Athias, M.

'19 Recherches sur les cellules interstitielles de l'ovaire des Cheiroptères. Arch. de Biol., T. XXX.

Boring, A. M.

'07 A Study of the Spermatogenesis of Twenty Species of the Membracidæ, Jassidæ, Cercopidæ and Fulgoridæ. Jour. Exp. Zoöl., Vol. IV., No. 4.

'13 The Chromosomes of the Cercopidæ. BIOL. BULL., Vol. XXIV., No. 3.

Browne, E. N.

'13 A Study of the Male Germ Cells in *Notonecta*. Jour. Exp. Zoöl., Vol. 14, No. 1.

'16 A Comparative Study of the Chromosomes of Six Species of *Notonecta*. Jour. Morph., Vol. 27, No. 1.

Buchner, P.

'09 Das accessorische chromosome in spermatogenese und ovogenese der Orthopteren, etc. Arch. f. Zellf. Bd. 3.

Calkins, G. N.

'95 Observations on the Yolk-nucleus in the Eggs of *Lumbricus*. Trans. N. Y. Acad. of Sci., Vol. XII.

Conklin, E. G.

'02 Karyokinesis and Cytokinesis, etc. Jour. Phila. A. N. S., Vol. 12.

'06 Sex Differentiation in *Dinophilus*. Science, Vol. XXIV., p. 294.

'05 The Organization and Cell-lineage of the Ascidian Egg. Jour. Phila. Acad., N.S., Vol. 13.

'17 The Share of Egg and Sperm in Heredity. Proc. Nat. Acad. Sci., Vol. 3.

'17 The effects of Centrifugal Force on the Eggs of Crepidula. Jour. Exp. Zoöl., Vol. 23.

Cowdry, E. V.

'16 The General Functional Significance of Mitochondria. Am. Jour. Anat., Vol. 19, No. 3.

Cowdry, N. H.

'17 A Comparison of Mitochondria in Plant and Animal Cells. BIOL. BULL., Vol. XXXIII., No. 3.

Crampton, H. E.

'99 Studies upon the Early History of the Ascidian Egg. Jour. Morph., Vol. 15, Supp.

Davis, H. S.

'08 Spermatogenesis in the Acridæ and Locustidæ. Bul. Mus. Comp. Zoöl. Harvard Coll., Vol. 53.

Duesberg, J.

'08 Sur l'existence des mitochondries dans l'oeuf et l'embryon d'Apis mellifica. Anat. Anzeig., Bd. XXXII.

'10 Nouvelles recherches sur l'appareil mitochondrial des cellules seminales. Arch. f. Zellf., Bd. 6, Heft. 1.

'12 Plastosomes, "Apparato reticolare interno," und Chromidial-apparat. Ergeb. der Anat. u. Entwick., Bd. XX.

'18 Chondriosomes in the Testicle-cells of *Fundulus*. Am. Jour. Anat., Vol. 23, No. 1.

'19 On the Present Status of the Chondriosome Problem. BIOL. BULL., Vol. XXXVI., No. 2.

Fauré-Frémiet, E.

'08 Evolution de l'appareil mitochondrial dans l'oeuf de Julus terrestris. C. R. Soc. de Biol. T. 64.

'10 Etude sur les mitochondries des Protozoaires et des cellules sexuelles. Arch. d'Anat. Micr. T. 11.

Foot, K. and Strobell, E. C.

'11 Amitosis in the Ovary of *Protenor belfragei* and a Study of the Chromatin Nucleolus. Arch. f. Zellf., Bd. 7, No. 2.

Gajewska, H.

'19 Ueber den sogenannten Dotterkern der Amphibien. Arch. f. Zellf. Bd. 15, 1st Heft.

Giardina, A.

'04 Sull 'esistenza di una speciale zona plasmatica perinucleare nell'oocite. Publicazioni de Laboratorio di Zoologica e. di Anat. Comp. dell Università di Palermo.

Goldschmidt, R.

'09 Das skelett der Muskelzelle von Ascaris nebst Bemerkungen ueber Chromidialapparat der Metazoenzelle. Arch. f. Zellf., Bd. IV.

Govaerts, P.

'13 Recherches sur la structure de l'ovaire des Insectes. Arch. de Biol., T. XXVIII.

Hegner, R. V.

'14 The Germ Cell Cycle in Animals. Macmillan, N. Y.

Kingsbury, B. F.

'12 Cytoplasmic Fixation. Anat. Rec., Vol. 6.

Kornhauser, S. I.

'14 A Comparative Study of the Chromosomes in the Spermatogenesis of *Enchenopa*, etc. Arch. f. Zellf., Bd. XII., No. 2.

Korschelt, E.

'86 Ueber die Enstehung und Bedeutung der verschiedenen Zellenelemente des Insektovariums. Zeit. f. Wiss. Zool., Bd. XLIII.

'89 Beitrage zur Morphologie und Physiologie des Zellkernes. Zool. Jahrb., Bd. 4.

Löwschin, A. M.

'13 "Myelinformen" und Chondriosomen. Ber. Deutsch. Bot. Gesell., Bd. 31.

Loyez, M.

'08 Les "noyaux de Blochmann" et la formation du vitellin chez les Hyménoptères. C. R. Assoc. Anat. 10 Réunion, Marseilles.

Maclean, H.

'18 Lecithin and Allied Substance. The Lipins. Monographs on Biochemistry, London.

McClung, C. E.

'14 A Comparative Study of the Chromosomes in Orthopteran Spermatogenesis. Jour. Morph., Vol. 25, No. 4.

'17 The Multiple Chromosomes of *Hesperotettix* and *Mermiria*. Jour. Morph., Vol. 29.

McGill, C.

'06 The Behavior of the Nucleoli during the Oögenesis of the Dragon-fly, etc. Zool. Jahrb., Bd. 23.

Marlatt, C. L.

'98 The Periodical Cicada. U. S. Dept. Agri. Div. Ent., Bull. 14, N.S.

Marshall, W. S.

'07a Contribution towards the Anatomy and Embryology of *Polistes pallipes*, II. Zeit. f. Wiss. Zool., Bd. 86.

'07b The Cellular Elements of the Ovary of *Platyphlax designatus*. Zeit. f. Wiss. Zool., Bd. 86.

Mathews, A. P.

'15 Physiological Chemistry. Wm. Wood and Co., N. Y., 1040 pp.

Montgomery, T. H.

'11 The Spermatogenesis of an Hemipteran, *Euschistus*. Jour. Morph., Vol. 22, No. 3.

Morgan, T. H.

'08 The Production of Two Kinds of Spermatozoa in the Phylloxerans, etc. Proc. Soc. Exp. Biol. and Med., Vol. 5, No. 3.

'09 A Biological and Cytological Determination of Sex Determination in Phylloxerans and Aphids. Jour. Exp. Zoöl., Vol. VII, No. 2.

Munson, J. P.

'99 The Ovarian Egg of *Limulus*. Jour. Morph., Vol. 15.

Nakahara, W.

'18 Some Observations on the Growing Oöcytes of the Stone-fly, *Perla immargenata* Say, etc. Anat. Rec., Vol. XV.

Payne, F. W.

'12 A Further Study of the Chromosomes of the Reduviidæ. Jour. Morph., Vol. XXIII.

'14 Chromosomal Variation and the Formation of the First Spermatocyte Chromosomes in the European Earwig, *Forficula* (sp.?). Jour. Morph., Vol. XXV., No. 4.

'16 A Study of the Germ Cells of *Gryllotalpa borealis*, etc. Jour. Morph., Vol. 28, No. 1.

Russo, A.

'12 Aumento dei granuli protoplasmici, etc. Arch. f. Zellf., Bd. 8.

Schafer, F.

'07 Spermatogenese von Dytiscus. Zool. Jahrb., Bd. 23.

Scott, W. J. M.

'16 Experimental Mitochondrial Changes in the Pancreas in Phosphorus Poisoning. Amer. Jour. Anat., Vol. 20, No. 2.

Shaffer, E. L.

'17 Mitochondria and other Structures in the Spermatogenesis of *Passalus cornutus*. BIOL. BULL., Vol. XXXII., No. 5.

'20 A Comparative Study of the Chromosomes of *Lachnosterna*. BIOL. BULL., Vol. 38, No. 2.

Stevens, N. M.

'05a A Study of the Germ Cells of *Aphis rosæ*, etc. Jour. Exp. Zoöl., Vol. 2, No. 3.

'05b Studies in Spermatogenesis with Especial Reference to the "Accessory" Chromosome. Carneg. Inst. Wash. Pub. No. 36, Part 2.

'06 Studies in Spermatogenesis. Part 2. Carneg. Inst. Wash. Pub. No. 36.

Sutton, W. S.

'02 On the Morphology of the Chromosome Groups of *Brachystola magna*. BIOL. BULL., Vol. 4.

Vander Stricht, O.

'98 Contribution à l'etude du noyau vitellin de Balbiani dans l'oocyte de la femme. Verhand. der Anat. Gesell., Jena.

'04 La couche vitellogène et les mitochondries de l'oeuf des Mammiferes. Verhand. der Anat. Gesell. Jena.

Vander Stricht, R.

'11 Vitellogénèse dans l'ovule de chatte. Arch. de Biol., T. XXVI.

Van Bambeke, Ch.

'95 Contribution à l'histoire de la constitution de l'oeuf. II. Arch. de Biol., T. XIII.

von Winwarter, H. et Sainmont, G.

'09 Nouvelles recherches sur l'ovogenèse et l'organogenèse de l'ovaire des Mammiferes (chat). Arch. de Biol., T. XXIV.

Wenrich, D. H.

'16 The Spermatogenesis of *Phryontettix magnus* with Especial Reference to Synapsis and the Individuality of the Chromosomes. Bull. Mus. Comp. Zoöl. Harvard Coll., Vol. LX., No. 3.

Wieman, H. L.

'10 A Study in the Germ Cells of *Leptinotarsa signaticollis.*, Jour. Morph., Vol. 21, No. 2.

Wilcox, E. V.

'95 Spermatogenesis of *Caloptenus femur-rubrum* and *Cicada tibicen.* Bull. Mus. Comp. Zoöl. Harvard Coll., Vol. XXVII.

Wildman, E. E.

'13 The Spermatogenesis of *Ascaris megalocephala* with Special Reference to Two Cytoplasmic Inclusions, etc. Jour. Morph., Vol. 24, No. 3.

Wilke, G.

'13 Chromatinreifung und Mitochondrienkorper in der spermatogeneses von *Hydrometra paludim.* Arch. f. Zellf., Bd. X.

Wilson, E. B.

'06 Studies on the Chromosomes, III. Jour. Exp. Zoöl., Vol. 3, No. 1.

'13 A Chromatoid Body in *Pentatoma.* BIOL. BULL., Vol. XXIV., No. 6.

ABBREVIATIONS.

AA, macrochromosome pair.
BB, CC, smaller chromosome pairs.
c., Centrosome.
cy., cytoplasm of oöcyte.
cr., chromatoid body.
cr.n., chromatin nucleolus of oöcyte.
e.s., egg-string of oöcyte.
f.e., follicular epithelium.
id., idiozome.
I.n.c., ingested nurse-cells.
N., Nebenkern of spermatid.
n.c., nurse-cell.
ncl., nucleus of oöcyte.
oocl., oöcyte.
pl., plasmosome.
S., spindle derivative.
X., sex-chromosome.

All drawings were made at table level with the aid of a camera lucida. Plates I, II, III, V (inc.) were made using a 1/12 oil immersion objective and a No. 12 ocular. They have been reduced ¼ in reproducing them here.

DESCRIPTION OF FIGURES.

PLATE I. (FIGS. 1 TO 12).

FIGS. 1, 2. Spermatogonial chromosome groups, showing macrochromosome pair (*AA*), the smaller chromosome pairs (*BB*, *CC*) and thirteen other chromosomes showing no size differences.

FIGS. 3, 4, 5, 6, 7, 8. Metaphase plates of follicle-cells of ovary, showing 20 chromosomes (female type). The chromosome pairs (*AA*, *BB*, *CC*) are easily distinguishable. Fig. 8 is late prophase.

FIG. 9. Metaphase plate from embryonic cell, showing 20 chromosomes hence of female type.

FIGS. 10, 11. Metaphase plates of ovarian follicle-cells (20 chromosomes) from follicles surrounding old oöcytes. Note that the chromosomes are poor in chromatin content; in Fig. 11 note the precocious longitudinal split of each chromosome.

FIG. 12. Late telophase of a follicle-cell division in which the macrochromosome, *A*, is recognizable in the daughter cells.

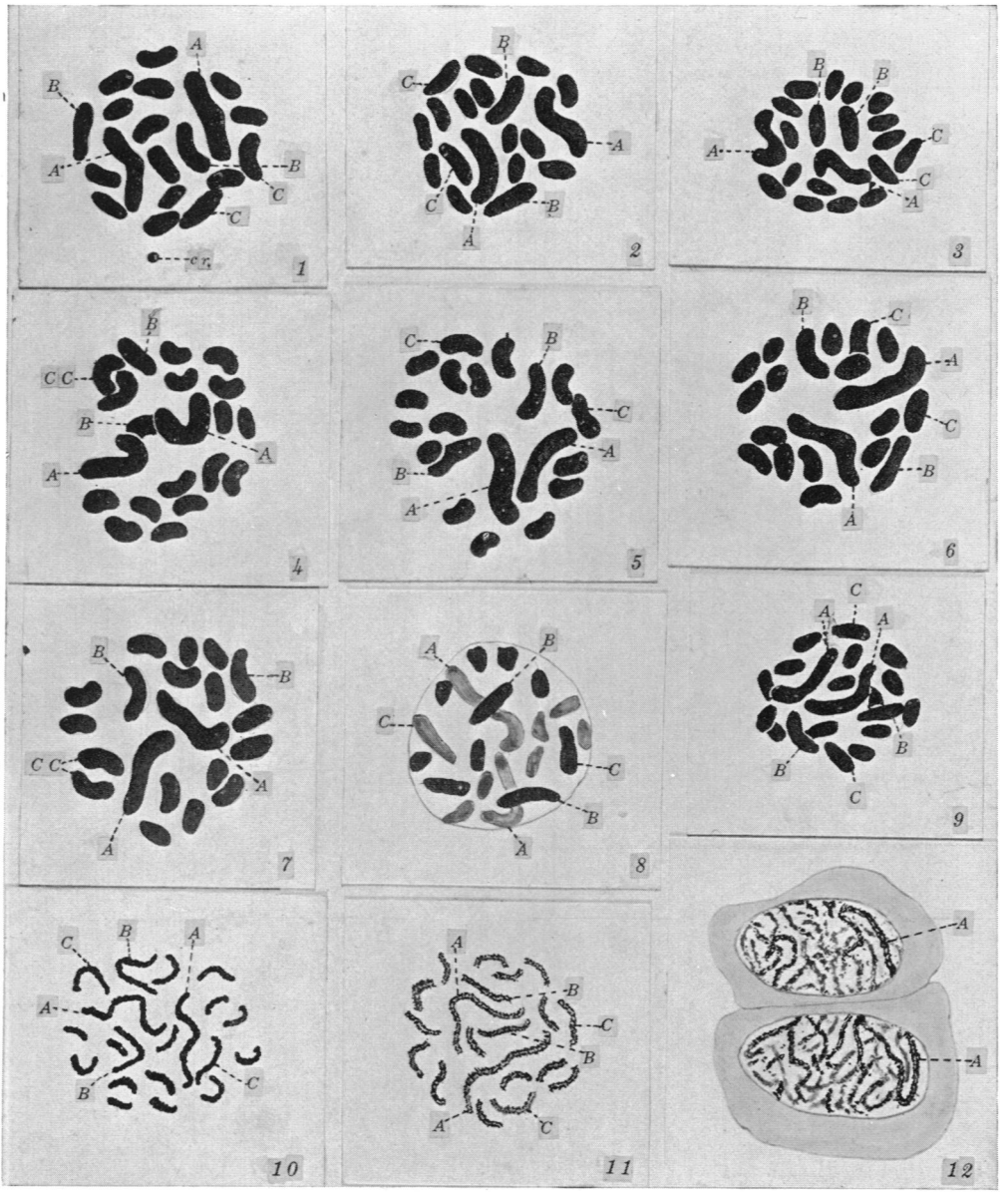

1
2
3
4
5
6
7
8
9
10
11
12

PLATE II. (FIGS. 13 TO 22).

FIG. 13. Secondary spermatogonium with characteristic chromatin nucleolus chromatoid body, and granular mitochondria localized at one end of the cell.

FIG. 14. Spermatogonium in early stage of degeneration. Mitochondria are larger due to agglutination, and nucleus is polymorphic.

FIGS. 15, 16. Later stages in degeneration of spermatogonia. Mitochondria continue to agglutinate and form large lipoid globules.

FIG. 17. Pachytene bouquet stage of spermatocyte, showing persisting sex-chromosome (X), the macrochromosome loop (AA) and the filar mitochondria localized about the idiozome (*id.*).

FIG. 18. Stages in the condensation of the ring tetrad of the macrochromosome pair (AA).

FIG. 19. Similar stages in the formation of the small ring tetrad of the BB chromosome pair.

FIG. 20. Early prophase of the first maturation division.

FIGS. 21, 22. Formation of first maturation spindle; mitochondria enveloping the spindle.

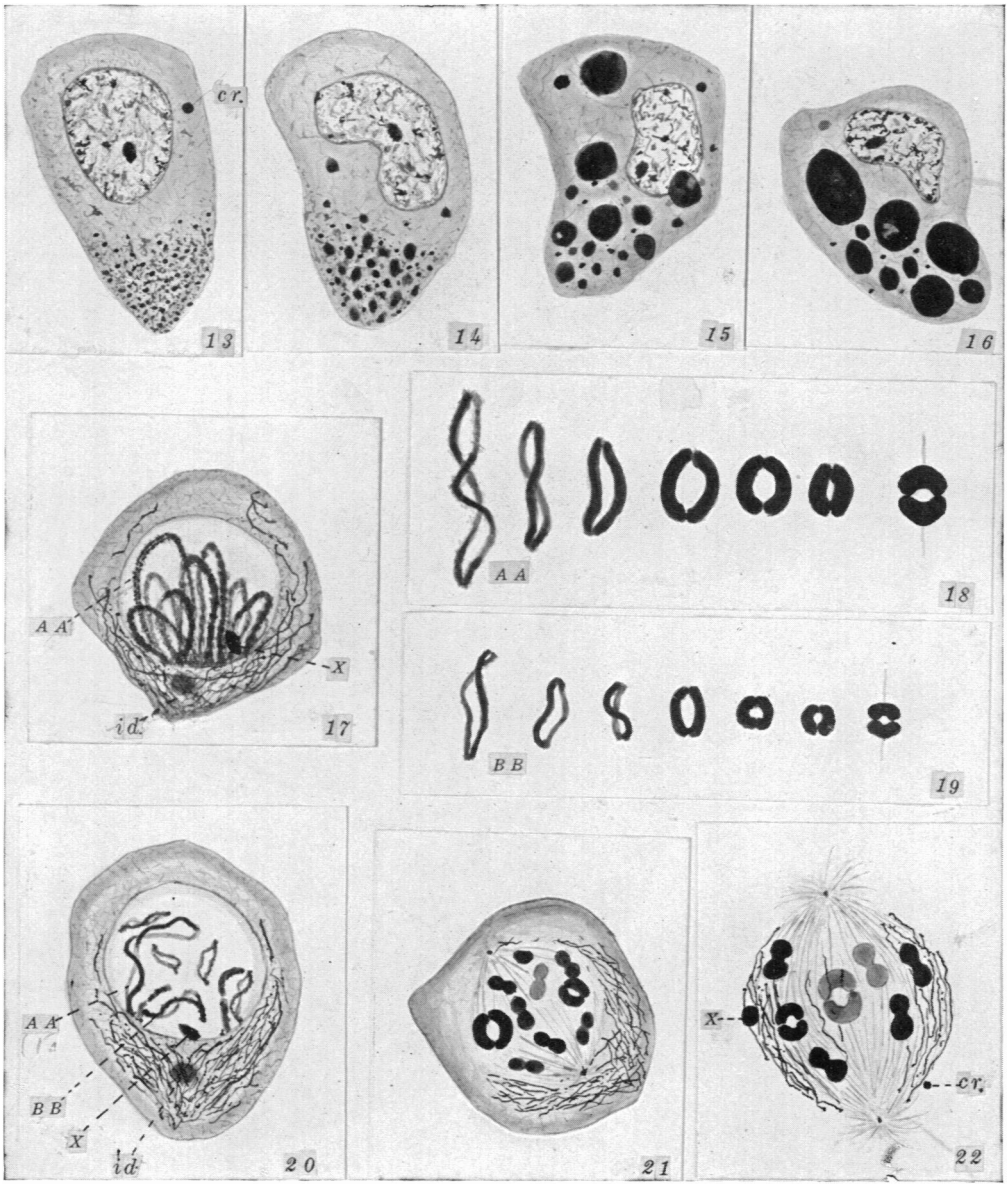

cr.
13
14
15
16
A A
X
id.
17
A A
18
B B
19
A A
B B
X
id
20
21
X
cr.
22

PLATE III. (FIGS. 23 TO 33).

FIGS. 23, 24. Metaphase plates of first spermatocytes showing characteristic grouping of chromosomes; macrochromosome tetrad in center surrounded by a circle of autosome tetrads with the sex-chromosome (*X*) lying outside the group.

FIGS. 25, 26. Successive sections of same cell. Anaphase of first maturation division showing separation of dyads of the *AA*, *BB*, *CC* tetrads. Mitochondria surrounding the spindle.

FIG. 27. Late anaphase of first maturation division. Sex-chromosome lags behind and appears bipartite; macrochromosome dyads (*A*, *A*) showing secondary split. Mitochondria are divided by cell-constriction.

FIG. 28. Daughter plates of second spermatocyte, one with 9 dyads the other with 9 dyads plus the sex-chromosome (*X*). All the dyads show the secondary split.

FIG. 29. Late anaphase of the second maturation division. Centrosomes adhere closely to the daughter nuclei; chromatoid body has passed to one of the daughter cells. Mitochondria are divided by the cell constriction.

FIG. 30. Normal spermatid. Centrosome (*c*) adheres closely to nuclear membrane.

FIG. 31. Giant spermatid, with all the structures of a normal spermatid, except that they are much larger.

FIG. 32. Transformation of the spermatid. Elongation of the Nebenkern pierced by axial filament. *Cr.*, chromatoid body passing out into tail.

FIG. 33. Stages in spermiogenesis. Elongation of Nebenkern to form sheath around axial filament.

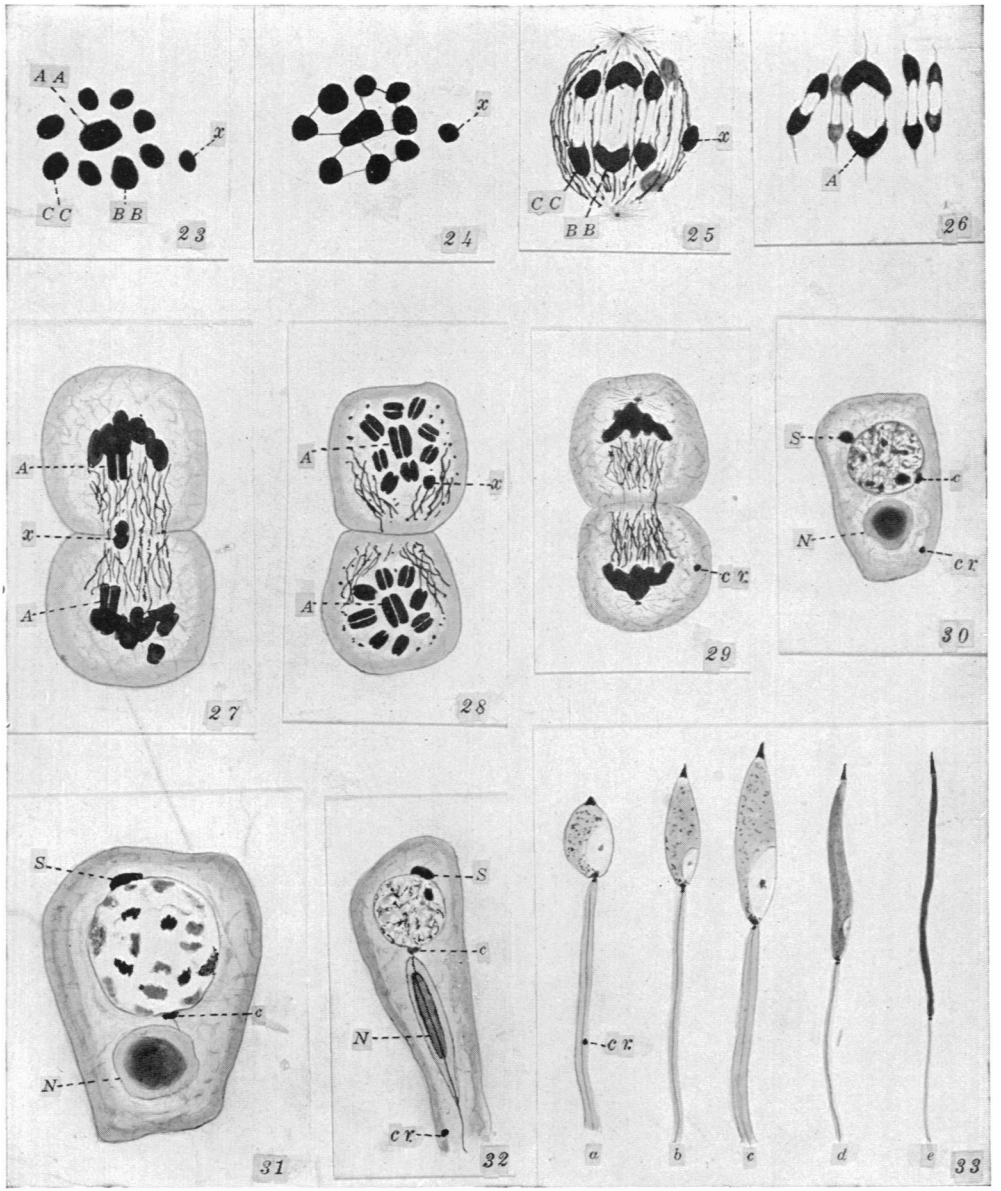

A A
x
C C
B B
23
x
24
x
C C
B B
25
A
26
A
x
A
27
A
x
A
28
c r
29
S
c
N
c r
30
S
c
N
31
S
c
N
c r
32
c r
a
b
c
d
e
33

PLATE IV. (FIG. 34).

FIG. 34. Longitudinal section through proximal end of ovarian tubule, × 500. Nurse-cells (*n.c.*) in nurse chamber with deeply staining cytoplasm. At base of nurse chamber are found young oöcytes in various stages of synapsis. *I.n.c.*, region where ingestion of nurse-cells takes place. Products of this ingestion are seen passing down egg-string (*e.s.*) to the older oöcyte (*ooct. 2*), which also shows a perinuclear zone of mitochondria.

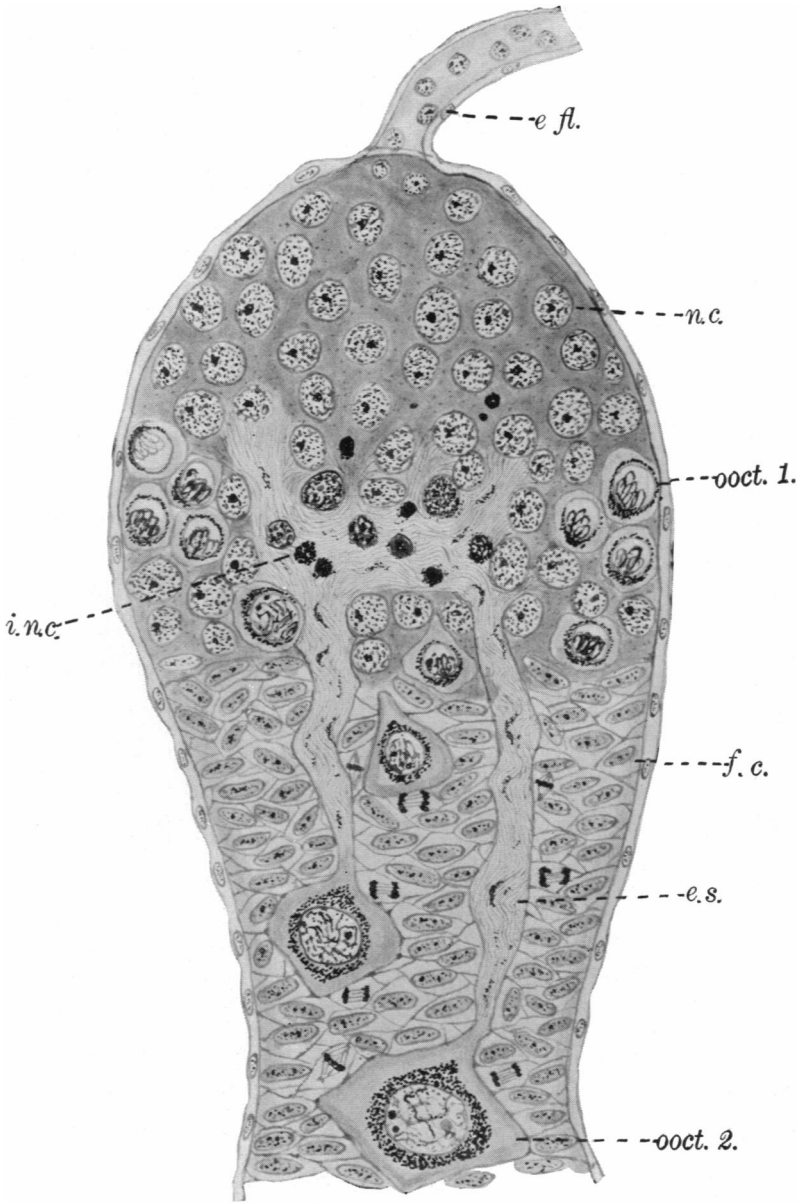

e fl.
n.c.
oct. 1.
i.n.c.
f. c.
e.s.
oct. 2.

PLATE V. (FIGS. 35 TO 43).

Synaptic Stages in the Oöcyte.

FIG. 35. Nucleus of very young oöcyte at beginning of growth period. Chromatin in form of a delicate network; two chromatin nucleoli are present (Protobroque nucleus).

FIG. 36. Deutobroque nucleus. Chromatin beginning to loose net-work appearance and individual threads become recognizable; two chromatin nucleoli present. Mitochondria in crescentic area closely applied to nuclear membrane.

FIG. 37. Leptotene stage. Polarization of threads; chromatic nucleoli absent Mitochondria spreading around nucleus.

FIG. 38. Zygotene stage. Leptotene threads are pairing parasynaptically. Mitochondria completely surround the nucleus.

FIG. 39. Pachytene stage. Bouin fixation, showing dissolution of mitochondria.

FIG. 40. Pachytene bouquet stage. Macrochromosome loop recognizable as largest loop.

FIG. 41. Section across bouquet stage showing pachytene threads on end view, 20 in number. Bouin fixation, showing effect on mitochondria.

FIG. 42. Release from bouquet stage; primary split evident in threads. Bouin fixation, 10 hours, showing artefacts produced by dissolution of mitochondria.

FIG. 43. Strepsistene stage. Synaptic threads separating and twisting about each other; two chromatin nucleoli are again present. Mitochondria increasing in numbers in perinuclear zone.

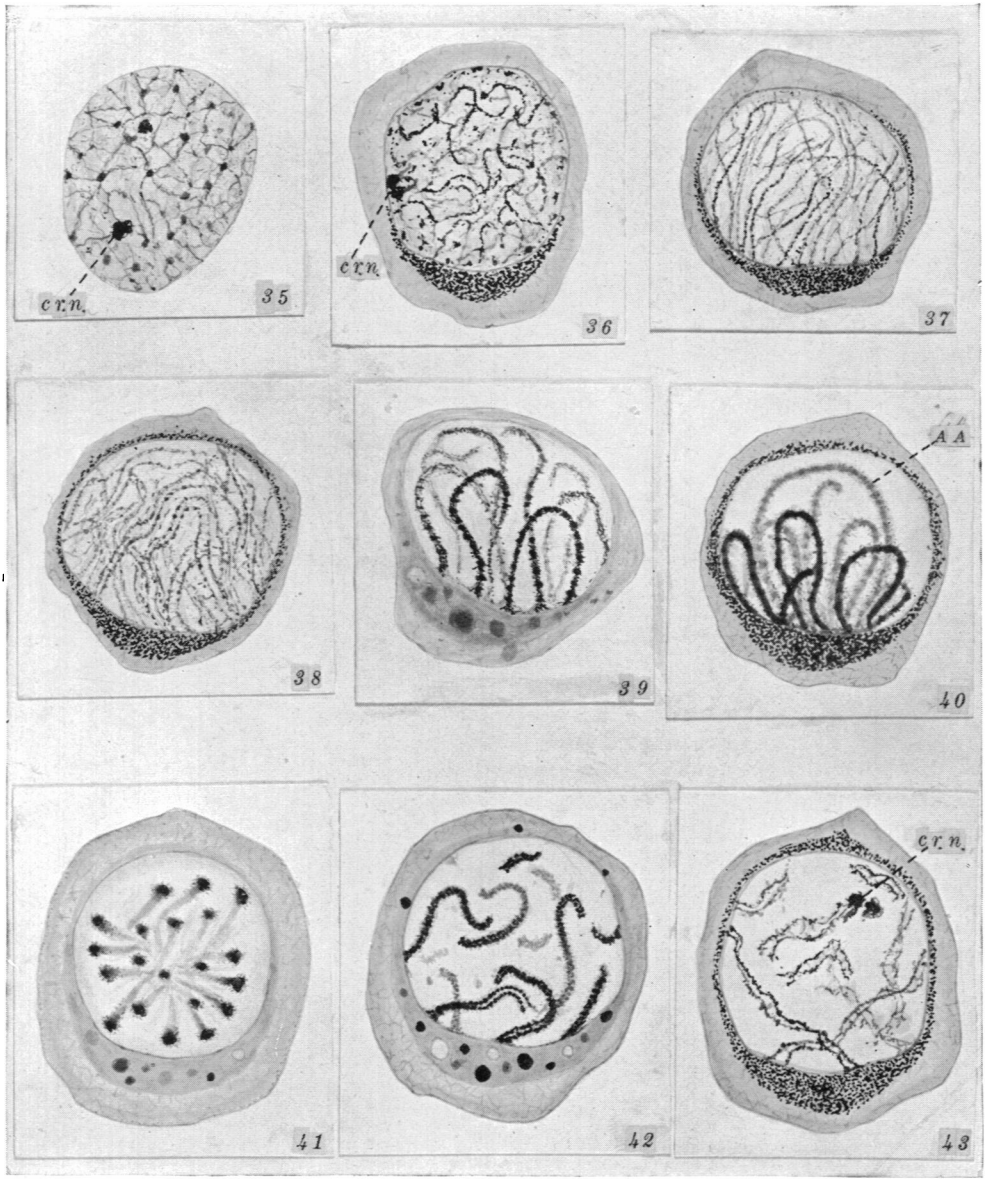

c.r.n.
35
c.r.n.
36
37
38
39
A A
40
c.r.n.
41
42
43

PLATE VI. (FIGS. 44 TO 48).

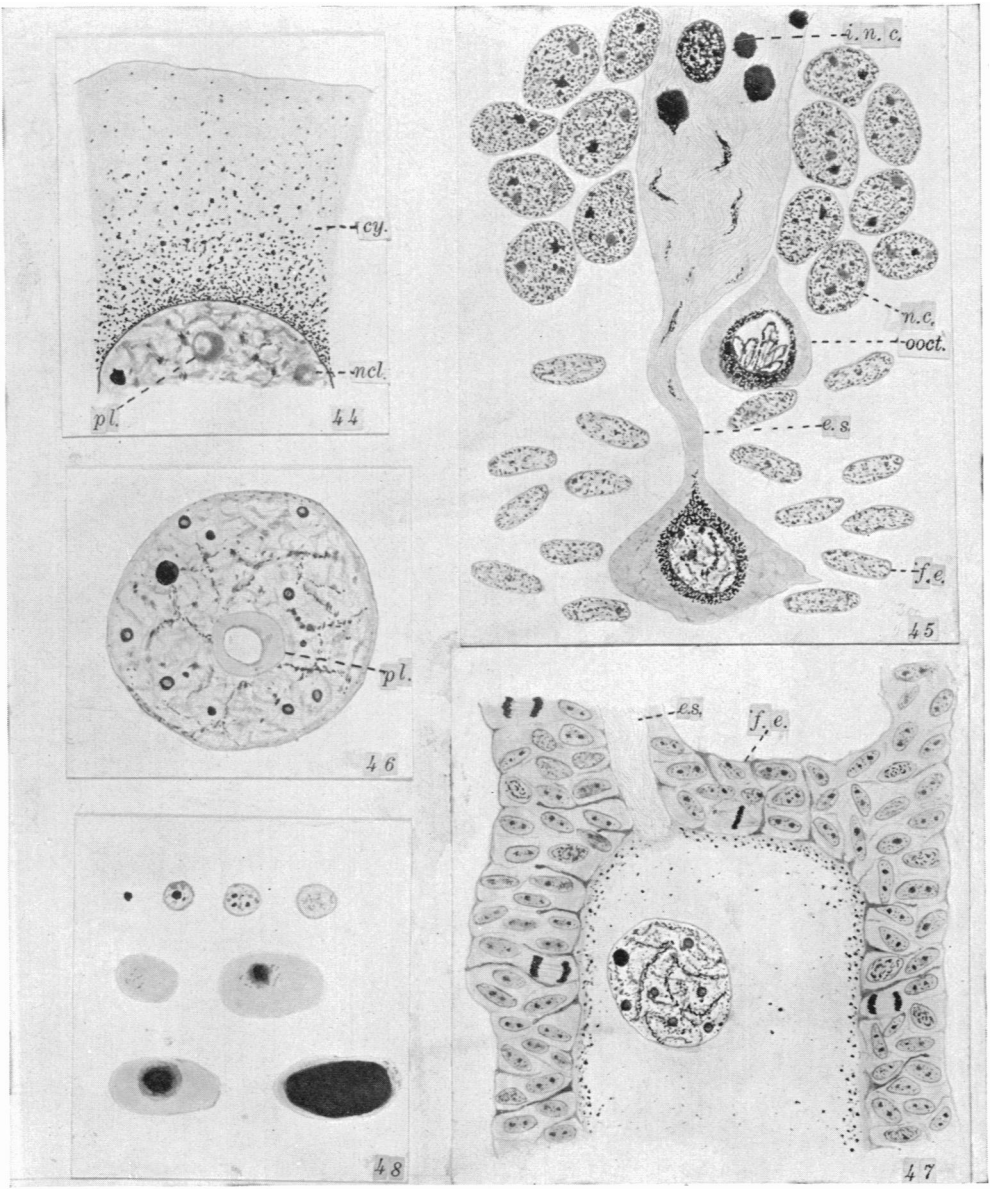
FIG. 44. Portion of nucleus (*ncl.*) and cytoplasm (*cy.*) of oöcyte showing migration of mitochondria from perinuclear region toward the periphery of the cell. × 1,200.

FIG. 45. Ingestion of nurse-cells by upper end of egg-string (*e.s.*). Products of ingestion are seen passing down egg-string into cytoplasm of oöcyte. Note perinuclear arrangement of mitochondria.

FIG. 46. Germinal vesicle of old oöcyte showing plasmosome (*pl.*) and several chromatic nucleoli. × 800.

FIG. 47. Portion of oöcyte and its follicle. Mitochondria arranged in periphery of cytoplasm. Persistence of egg-string. × 600.

FIG. 48. Stages in the transformation of mitochondria into yolk-sperules.


cy.
ncl.
pl.
44
i. n. c.
n.c.
ooct.
e. s.
f. e.
45
pl.
46
e.s.
f. e.
47
48

PLATES VII, VIII, IX.

Photomicrographs taken at a magnification of about 1,000 diameters, except Nos. 71 and 72 which have been magnified 500. The reproductions here have not been reduced.

PLATE VII. (FIGS. 49 TO 70).

FIGS. 49, 50, 51. Metaphase plates of follicle-cells of ovary, showing 20 chromosomes among which the macrochromosome pair can be distinguished. Fig. 51 is at a greater magnification.

FIG. 52. Tripolar spindle in one of the cells of the adhesive gland in the female.

FIGS. 53, 54, 55, 56. Metaphase plates of first spermatocytes from four different animals, each showing 10 bivalent chromosomes which are similarly grouped in each case.

FIG. 57. An oblique section through the metaphase plate of the 1st maturation division, showing the two halves of the macrochromosome in the center of the complex.

FIG. 58. Daughter plates of second spermatocytes, one with 9 dyads, the other with 9 dyads plus the sex-chromosome. Note that the grouping of the chromosomes is the same as in the first spermatocyte.

FIG. 59. Giant spermatocyte with a great many bivalent chromosomes and a large amount of mitochondria.

FIG. 60. Anaphase of first maturation division showing mitochondria surrounding spindle.

FIGS. 61, 62, 63. Various forms of ring tetrad (macrochromosome tetrad) in the early prophases.

FIG. 64. Macrochromosome tetrad in late prophase.

FIG. 65. Late prophase of first spermatocyte, showing character of tetrads.

FIG. 66. Anaphase of first maturation division from smear preparation, showing separation of macrochromosome dyads.

FIG. 67. Ring tetrad in prophase of first spermatocyte; from smear preparation.

FIG. 68. First maturation division showing separation of macrochromosome dyads.

FIG. 69. Anaphase of first maturation division showing separation of the dumb-bell shaped tetrads.

FIG. 70. Late anaphase of first maturation division, showing lagging sex-chromosome.

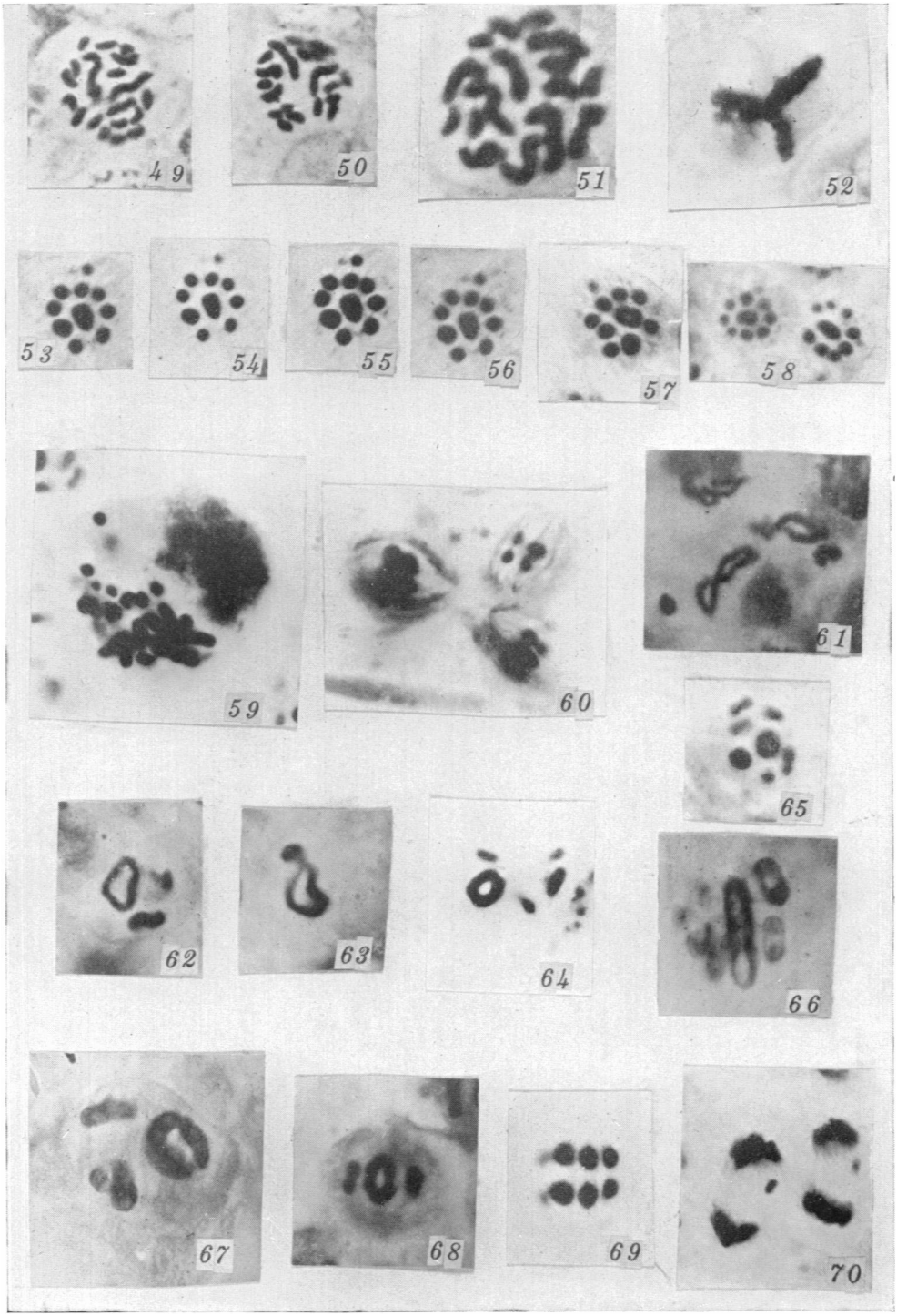

49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70

PLATE VIII. (FIGS. 71 TO 81).

FIGS. 71, 72. Portions of the nurse chambers of the ovaries, showing fibrous appearance of central plasmatic mass in which the egg-strings of the oöcytes end. Nurse-cells in various stages of ingestion may be seen and the products of their disintegration may be seen passing down the egg-strings into the oöcytes. The oöcytes are distinguishable by their deeply staining perinuclear zone of mitochondria.

Figs. 73 to 75 are various stages of synapsis in oöcyte.

FIG. 73. Protobroque nucleus at beginning of synaptic period. Two chromatic nucleoli are present.

FIG. 74. Deutobroque nucleus of oöcyte. Treads become more evident; two chromatic nucleoli are present.

FIG. 75. Leptotene stage. Polarization of the threads. Chromatic nucleoli are absent.

FIG. 76. Pachytene bouquet stage.

FIG. 77. Section across bouquet stage, showing 20 threads on end view.

FIG. 78. Strepsistene stage. Reappearance of the two chromatin nucleoli.

FIG. 80. Oöcyte from ovaries fixed in Bouin's fluid (10 hours) showing effect of acetic acid on mitochondria. Vacuoles, globules and nucleolar-like structures are found in cytoplasm due to the partial dissolution and agglutination of the mitochondria.

FIG. 81. Oöcyte after fixation in Bouin's fluid for six hours, showing perinuclear zone of mitochondria only partially destroyed. Two chromatin nucleoli are present in the nucleus.

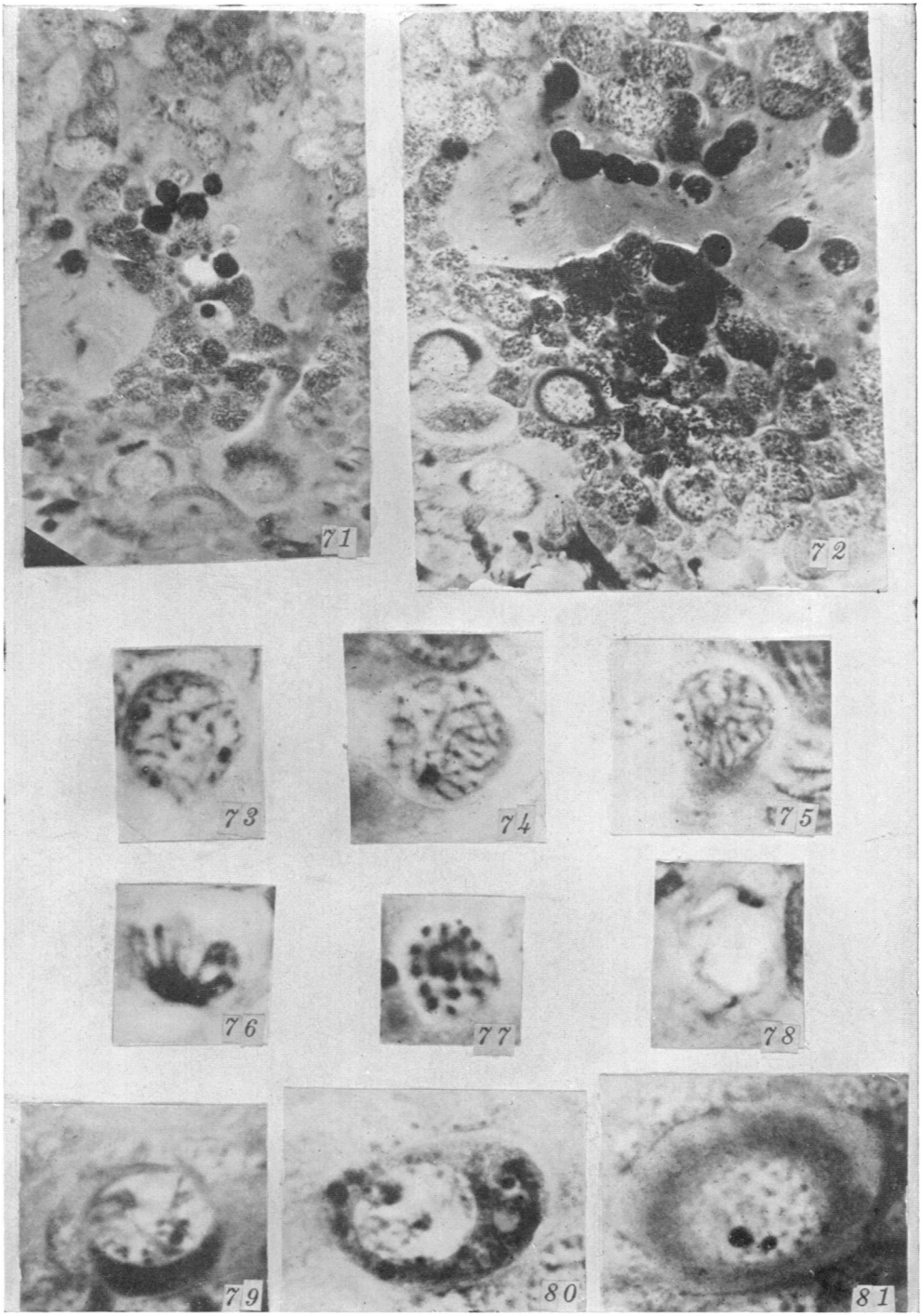

71
72
73
74
75
76
77
78
79
80
81

PLATE IX. (FIGS. 82 TO 87).

FIG. 82. Oöcyte with its egg-string, from ovaries fixed in Bouin's fluid 15 hours showing the complete disappearance of mitochondria.

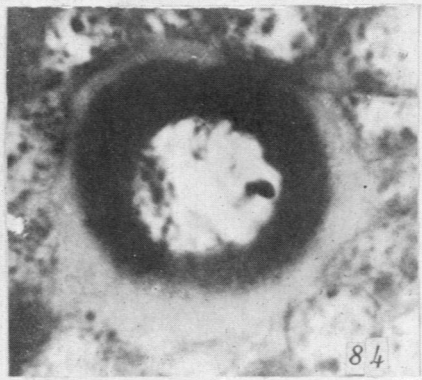
FIGS. 83, 84. Oöcytes from Flemming fixed material showing characteristic arrangement of mitochondria in a perinuclear zone sharply delimited from the rest of the cytoplasm.

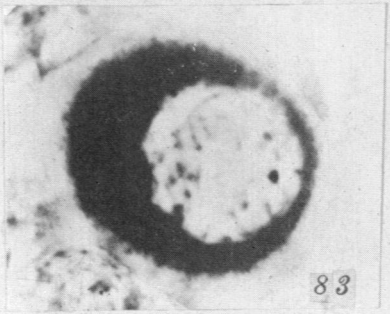
FIG. 85. Oöcyte at the period when the cytoplasmic volume has reached its maximum. The mitochondria still surround the nucleus, but the zone is not so sharply delimited. Shrinkage of the nucleus away from the cytoplasm shows the nuclear membrane to be intact.

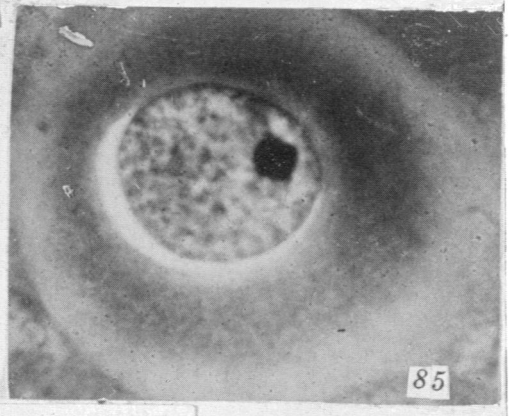
FIG. 86. Older oöcyte in which perinuclear arrangement of mitochondria is lost, the granules becoming scattered toward the periphery of the cytoplasm.

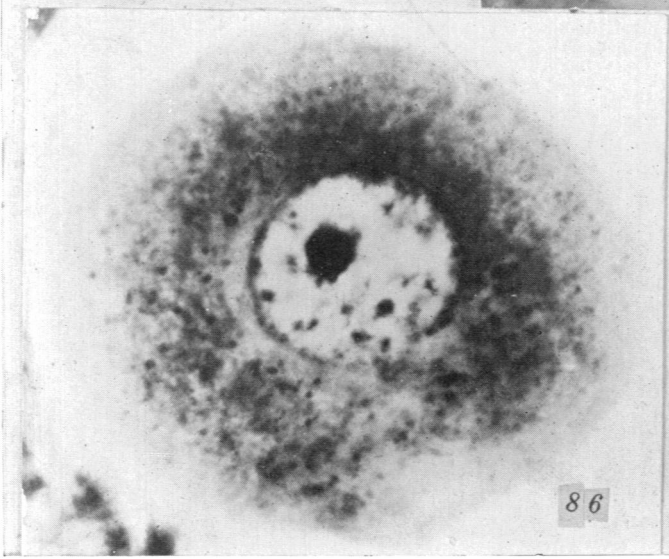
FIG. 87. Typical germinal vesicle of almost mature oöcyte, showing two chromatic nucleoli.

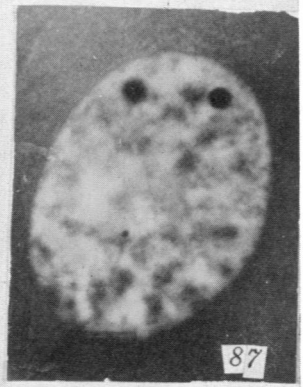

82


84


83


85


86


87